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(54) Title: COMPOSITIONS AND METHODS FOR TREATING AND DIAGNOSING ASTHMA

(57) Abstract: Compositions, kits and methods for treating and diagnosing subtypes of asthma patients are provided. Also provided are methods for identifying effective asthma therapeutic agents and predicting responsiveness to asthma therapeutic agents.

COMPOSITIONS AND METHODS FOR TREATING AND DIAGNOSING ASTHMA

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims the benefit of U.S. provisional application numbers 61/072,572 filed 31 March 2008, 61/041,480 filed 1 April 2008, 61/128,383 filed 20 May 2008, and 61/205,392 filed 16 January 2009.

FIELD

[0002] Compositions and methods for treating and diagnosing subtypes of asthma patients are provided. Also provided are methods for identifying effective asthma therapeutic agents and predicting responsiveness to asthma therapeutic agents.

BACKGROUND

[0003] Asthma is traditionally thought to result from aeroallergen-induced inflammation driven by T-helper type 2 (Th2) processes and mediated by cytokines including interleukin (IL)-4, IL-5 and IL-13. IL-13 is a pleiotropic Th2 cytokine produced by activated T cells, basophils, eosinophils, and mast cells, and it has been strongly implicated in the pathogenesis of asthma in preclinical models [2]. Elevated levels of IL-13 have been detected in the airways of human asthma patients; however, this elevation is only observed in a subset of asthmatics [3-6]. Recent research has been directed at understanding how Th2 cytokines cause asthma-like pathology and physiology [49, 50].

[0004] While asthma is often characterized by eosinophilic infiltration of the airways, there is increasing evidence that there are other subtypes of the disease driven by alternative forms of inflammation [1, 39, 48]. For example, studies of the cellular components of airway inflammation in asthma provide evidence for distinct eosinophilic and non-eosinophilic phenotypes of asthma [1, 39, 48]. Whether the molecular mechanisms underlying these clinical and cellular phenotypes of asthma differ is unknown. The identification of and development of biomarkers for distinct molecular phenotypes of asthma would guide the direction of basic research and the clinical application of emerging asthma therapies that specifically target Th2 responses in the lung.

[0005] Periostin is a secreted protein associated with fibrosis whose expression is upregulated by recombinant IL-4 and IL-13 in bronchial epithelial cells [7, 8] and bronchial fibroblasts [9]. It is expressed at elevated levels in vivo in bronchial epithelial cells [8] and in the subepithelial bronchial layer [9] of human asthmatics as well as in a mouse model of asthma [10]. It is also expressed at elevated levels in the esophageal epithelium of patients with eosinophilic esophagitis in an IL-13 dependent manner [11]. Elevated periostin expression has been observed in several types of epithelial derived cancers [64-67], and elevated levels of soluble periostin have been observed in the serum of some cancer patients [64, 68-70].

[0006] Genome-wide expression microarray analyses of bronchial epithelial cells from 42 mild-to-moderate, steroid-naïve asthmatics and 28 healthy control subjects have been performed [8]. In those studies, three of the most differentially expressed epithelial genes between all asthmatics and all healthy controls were periostin, CLCA1, and serpinB2 [8]. Furthermore, those genes were significantly downregulated in bronchial epithelial cells of asthmatics after 7 days of inhaled corticosteroid (ICS) treatment [8]. All three of those genes are induced in bronchial epithelial cells by recombinant IL-13 treatment in vitro and their expression is markedly attenuated by addition of corticosteroids to the cell culture medium [8].

[0007] To date, such genome-wide expression analyses have not identified genetic biomarkers that are prognostic or predictive of therapeutic response to treatment for individual asthma patients, nor have they identified genetic biomarkers that distinguish subtypes of asthmatic patients. In addition, no reliable nongenetic biomarkers with broad clinical applicability for prognostic or predictive responses to therapeutic treatment, or diagnostic of subtypes of asthma, have been identified. Thus, as asthma patients seek treatment, there is considerable trial and error involved in the search for therapeutic agent(s) effective for a particular patient. Such trial and error often involves considerable risk and discomfort to the patient in order to find the most effective therapy.

[0008] Thus, there is a need for more effective means for determining which patients will respond to which treatment and for incorporating such determinations into more effective treatment regimens for asthma patients.

[0009] The invention described herein meets the above-described needs and provides other benefits.

SUMMARY

[0010] Using gene expression signatures in bronchial epithelium, we have defined distinct molecular subtypes of asthma. Surprisingly, supervised clustering of the data based on a set of genes whose expression was highly correlated to genes known to be upregulated by IL-4 or IL-13 stimulation revealed not one but two distinct clusters of asthma patients. Furthermore, analysis of these dichotomous subsets of asthmatics revealed significant associations between "IL-4/13 signature" status and serum total IgE levels, serum CEA levels, serum periostin levels, peripheral blood eosinophilia, (bronchoalveolar lavage) BAL eosinophilia, and responsiveness to inhaled corticosteroids (each $p < 0.05$ by Wilcoxon rank sum test).

[0011] Accordingly, the present invention relates to methods of diagnosing a subpopulation of asthma patients comprising measuring the gene expression of any one or combination of genes selected from POSTN, CST1, CCL26, CLCA1, CST2, PRR4, SERPINB2, CEACAM5, iNOS, SERPINB4, CST4, PRB4, TPSD1, TPSG1, MFSD2, CPA3, GPR105, CDH26, GSN, C2ORF32, TRACH2000196 (TMEM71), DNAJC12, RGS13, SLC18A2, SERPINB10, SH3RF2, FCER1B, RUNX2, PTGS1, and ALOX15. In one embodiment, the gene expression is measured of any one or combination of genes selected from the group of consisting of POSTN, CST1, CST2, CCL26, CLCA1, PRR4, PRB4, SERPINB2, CEACAM5, iNOS, SERPINB4, CST4, and SERPINB10. According to one embodiment, the gene expression is measured by microarray. According to another embodiment, the gene expression is measured by observing protein expression levels of an aforementioned gene. According to another embodiment, the gene expression is considered elevated when compared to a healthy control if the relative mRNA level of the gene of interest is greater than 2.5 of the level of a control gene mRNA. According to another embodiment, the relative mRNA level of the gene of interest is greater than 3 fold, 5 fold, 10 fold, 15 fold 25 fold or 30 fold compared to a healthy control gene expression level. According to one embodiment, the gene expression is measured by a method selected from the group consisting of a PCR method, a microarray method or a immunoassay method. In one embodiment, the microarray method comprises the use of a microarray chip having one or more nucleic acid molecules that can hybridize under stringent conditions to a nucleic acid molecule encoding a gene mentioned above or having one or more polypeptides (such as peptides or antibodies) that can bind to one or more of the proteins encoded by the genes mentioned above. In one embodiment, the PCR method is qPCR. According to one

embodiment, the immunoassay method comprises the steps of binding an antibody to protein expressed from a gene mentioned above in a patient sample mentioned above and determining if the protein level from the patient sample is elevated. According to one embodiment, a control gene is a housekeeping gene selected from the group consisting of actin, GAPDH, GASB and GUSB.

[0012] The present invention provides a microarray chip comprising nucleic acid sequences encoding the following genes: POSTN, CST1, CST2, CCL26, CLCA1, PRR4, SERPINB2, CEACAM5, iNOS, SERPINB4, CST4, and SERPINB10 or fragments thereof. The present invention provides a microarray chip comprising nucleic acid sequences encoding the following genes: POSTN, CST1, CCL26, CLCA1, CST2, PRR4, SERPINB2, CEACAM5, iNOS, SERPINB4, CST4, PRB4, TPSD1, TPSG1, MFSD2, CPA3, GPR105, CDH26, GSN, C2ORF32, TRACH2000196 (TMEM71), DNAJC12, RGS13, SLC18A2, SERPINB10, SH3RF2, FCER1B, RUNX2, PTGS1, and ALOX1, or fragments thereof.

[0013] The present invention provides a subpopulation of asthma patients to be treated with the therapeutic agents of this invention, wherein the ratio of Muc5AC:MUC5B protein or mRNA levels in the airway epithelial cells of asthma patients is greater than 25.

[0014] The present invention also relates to methods of diagnosing a subpopulation of asthma patients by taking single or combinations of measurements of systemic biomarkers selected from serum CEA levels, serum IgE levels, serum periostin levels, peripheral blood eosinophil counts and eosinophil percentages in bronchoalveolar lavage fluid (BAL). Systemic biomarkers typically are nongenetic biomarkers and are typically measured in samples obtained by noninvasive procedures, for example, but not limited to, collection of blood or blood components, e.g., serum or plasma. According to one embodiment, greater than 100IU/ml IgE levels and/or $0.14 \times 10^9/L$ eosinophils is predictive of a patient population to be treated with the therapeutic agents of this invention.

[0015] The present invention relates to methods of treating asthma comprising administering a therapeutic agent to a patient expressing elevated levels of any one or combination of the genes selected from POSTN, CST1, CCL26, CLCA1, CST2, PRR4, SERPINB2, CEACAM5, iNOS, SERPINB4, CST4, PRB4, TPSD1, TPSG1, MFSD2, CPA3, GPR105, CDH26, GSN, C2ORF32, TRACH2000196 (TMEM71), DNAJC12, RGS13, SLC18A2, SERPINB10, SH3RF2, FCER1B, RUNX2, PTGS1, ALOX15. According to one embodiment, the patient expresses elevated levels of any one or combination of genes selected from the group consisting of periostin, CST1, CST2, CCL26, CLCA1, PRR4,

SerpinB2, CEACAM5, iNOS, PRB4, SerpinB4, SerpinB10 and CST4. According to one embodiment, the patient to be treated is a mild-to-moderate, steroid-naïve (never treated with steroids) asthma patient. According to another embodiment, the patient to be treated is a moderate-to-severe, steroid-resistant (non-responsive to steroids) asthma patient. Such patients are treated with a therapeutically effective amount of a therapeutic agent. In one embodiment, the patient has asthma induced by the TH2 pathway.

[0016] According to one embodiment, the therapeutic agent is an anti-IL13/IL4 pathway inhibitor. According to another embodiment, the therapeutic agent targets the TH2 induced asthma pathway. Exemplary targets include, but are not limited to, cytokines or ligands such as: IL-9, IL-5, IL-13, IL-4, OX40L, TSLP, IL-25, IL-33 and IgE; and receptors such as: IL-9 receptor, IL-5 receptor, IL-4receptor alpha, IL-13receptoralpha1 and IL-13receptoralpha2, OX40, TSLP-R, IL-7Ralpha (a co-receptor for TSLP), IL17RB (receptor for IL-25), ST2 (receptor for IL-33), CCR3, CCR4, CRTH2, FcepsilonRI and FcepsilonRII/CD23 (receptors for IgE). Accordingly, a therapeutic agent according to this invention includes an agent that can bind to the target above, such as a polypeptide(s) (e.g., an antibody, an immunoadhesin or a peptibody), an aptamer or a small molecule.

[0017] According to one embodiment, the therapeutic agent is an anti-IL13 antibody. According to another embodiment, the anti-IL-13 antibody comprises a VH sequence comprising SEQ ID NO: 193 and a VL sequence comprising SEQ ID NO:194. According to another embodiment, the anti-IL13 antibody comprises: (a) an HVR-L1 comprising amino acid sequence RASKSVDSYGNSFMH (SEQ ID NO:195); (b) an HVR-L2 comprising amino acid sequence LASNLES (SEQ ID NO:196); (c) an HVR-L3 comprising amino acid sequence QQNNEDPRT (SEQ ID NO: 197); (d) an HVR-H1 comprising amino acid sequence AYSVN (SEQ ID NO:198); (e) an HVR-H2 comprising amino acid sequence MIWGDGKIVYNSALKS (SEQ ID NO: 199); and (f) an HVR-H3 comprising amino acid sequence DGYYPYAMDN (SEQ ID NO: 200). According to another embodiment, the therapeutic agent is an anti-OX40 ligand (OX40L) antibody. According to another embodiment the therapeutic agent is an anti-IL13/anti-IL4 bispecific antibody. According to another embodiment, the therapeutic agent is an anti-IgE antibody. According to another embodiment, the therapeutic agent is an antibody directed against the membrane proximal M1' region of surface expressed IgE on B cells. According to another embodiment, the therapeutic agent is an inhaled corticosteroid. In certain embodiments, the inhaled

corticosteroid is selected from beclomethasone dipropionate, budesonide, flunisolide, fluticasone propionate, mometasone, and triamcinolone acetonide.

[0018] According to one embodiment, the anti-OX40L antibody comprises: (a) an HVR-L1 comprising sequence RSSQSPVHSNGNTYLH (SEQ ID NO:201); (b) an HVR-L2 comprising sequence KVSNRFS (SEQ ID NO: 202); (c) an HVR-L3 comprising sequence SQSTHIPWT (SEQ ID NO: 203); (d) an HVR-H1 comprising sequence SYWMH (SEQ ID NO: 204); (e) an HVR-H2 comprising sequence EIDPSNGRTNYNEKFKS (SEQ ID NO: 205); and (f) an HVR-H3 comprising sequence ERSPLYFDV (SEQ ID NO:206).

According to another embodiment, the anti-OX40L antibody comprises: (a) an HVR-L1 comprising sequence RSSQSIVHNGNTYLE (SEQ ID NO:207); (b) an HVR-L2 comprising sequence RVSNRFS (SEQ ID NO:208); (c) an HVR-L3 comprising sequence FQGSHPYPT (SEQ ID NO:209); (d) an HVR-H1 comprising sequence SYWLN (SEQ ID NO:210); (e) an HVR-H2 comprising sequence MIDPSDSETHYNQVFKD (SEQ ID NO:211); and (f) an HVR-H3 comprising sequence GRGNFYGGSHAMEY (SEQ ID NO:212). According to another embodiment, the anti-OX40L antibody comprises (a) an HVR-H1 comprising sequence SYTMH (SEQ ID NO:215), SYAMS (SEQ ID NO:216), NFGMH (SEQ ID NO:217), or NYGMH (SEQ ID NO:218), (b) an HVR- H2 comprising sequence IISGSGFTYYADSVKG (SEQ ID NO:219), AIWYDGHDKYYSYVYVKG (SEQ ID NO:220), AIWYDGHDKYYAYVYVKG (SEQ ID NO:221), VIWYDGSNKYYVDSVKG (SEQ ID NO:222), or VIWNDGSNKYYVDSVKG (SEQ ID NO:223), (c) an HVR-H3 comprising sequence DSSSWYRYFDY (SEQ ID NO:224), DRLVAPGTFDY (SEQ ID NO:225), KNWSFDF (SEQ ID NO:226), or DRMGIIYYGMDV (SEQ ID NO:227), (d) an HVR-L1 comprising sequence RASQGISSWLA (SEQ ID NO:228), RASQSVSSSYLA (SEQ ID NO:229), RASQSVSSNYLA (SEQ ID NO:230), RASQGVSRYLE (SEQ ID NO:231), or RASQSVSSSYLA (SEQ ID NO:232), (e) an HVR- L2 comprising sequence GASSRAT (SEQ ID NO:233), AASSLQS (SEQ ID NO:234), MPPVWKV (SEQ ID NO:235), DASNRAT (SEQ ID NO:236), or LHPLCKV (SEQ ID NO:237); and (f) an HVR- L3 comprising sequence NSLIVTLT (SEQ ID NO:238), QQYNSYPYT (SEQ ID NO:239), QQYGSST (SEQ ID NO:240), QQRSNWQYT (SEQ ID NO:241), QQRSNWT (SEQ ID NO:242), or NSIIVSLT (SEQ ID NO:243), wherein the anti-OX40L antibody binds OX40L. According to one embodiment, the anti-IgE antibody comprises a VL sequence comprising SEQ ID NO:213 and a VH sequence comprising SEQ ID NO:214. According to another

embodiment, the anti-IgE antibody comprises: (a) an HVR-L1 comprising sequence RSSQSLVHNNANTYLH (SEQ ID NO:244) (b) an HVR-L2 comprising sequence KVSNNRFS (SEQ ID NO: 245); (c) an HVR-L3 comprising sequence SQNTLVPWT (SEQ ID NO: 246); (d) an HVR-H1 comprising sequence GFTFSDYGIA (SEQ ID NO: 247); (e) an HVR-H2 comprising sequence AFISDLAYTIYYADTVTG (SEQ ID NO: 248); and (f) an HVR-H3 comprising sequence ARDNWDAMDY (SEQ ID NO:249). According to one embodiment, the anti-IgE antibody comprises a VH sequence comprising SEQ ID NO:250 and a VL sequence comprising SEQ ID NO:251. According to one embodiment, the anti-IgE antibody comprises a VH sequence comprising SEQ ID NO:252 and a VL sequence comprising SEQ ID NO:253. According to another embodiment, the anti-IgE antibody comprises: (a) an HVR-L1 comprising sequence RSSQDISNSLN (SEQ ID NO:254) (b) an HVR-L2 comprising sequence STSRLHS (SEQ ID NO: 255); (c) an HVR-L3 comprising sequence QQGHTLPWT (SEQ ID NO: 256); (d) an HVR-H1 comprising sequence GYTFTDYYMM (SEQ ID NO: 257); (e) an HVR-H2 comprising sequence GDNIDPNNYDTSYNQKFKG (SEQ ID NO: 258); and (f) an HVR-H3 comprising sequence ASKAY (SEQ ID NO:259). According to another embodiment, the anti-IgE antibody comprises: (a) an HVR-L1 comprising sequence RSSQDISNALN (SEQ ID NO:260) (b) an HVR-L2 comprising sequence STSRLHS (SEQ ID NO: 255); (c) an HVR-L3 comprising sequence QQGHTLPWT (SEQ ID NO: 256); (d) an HVR-H1 comprising sequence GYTFTDYYMM (SEQ ID NO: 257); (e) an HVR-H2 comprising sequence GDNIDPNNYDTSYNQKFKG (SEQ ID NO: 258); and (f) an HVR-H3 comprising sequence ASKAY (SEQ ID NO:259). According to another embodiment, the anti-IgE antibody comprises: (a) an HVR-L1 comprising sequence RSSQDISNALN (SEQ ID NO:260) (b) an HVR-L2 comprising sequence STSRLHS (SEQ ID NO: 255); (c) an HVR-L3 comprising sequence QQGHTLPWT (SEQ ID NO: 256); (d) an HVR-H1 comprising sequence GYTFTDYYIM (SEQ ID NO: 261); (e) an HVR-H2 comprising sequence GDNIDPNNYDTSYNQKFKG (SEQ ID NO: 258); and (f) an HVR-H3 comprising sequence ASKAY (SEQ ID NO:259).

[0019] According to one embodiment, the patient has asthma that does not involve the TH2 pathway (non-TH2 asthma). In one embodiment, the therapeutic agent targets non-TH2 asthma. According to one embodiment, the therapeutic agent is an IL-17 pathway inhibitor. In one embodiment, the therapeutic agent is anti-IL-17 antibody. In one embodiment, the therapeutic agent is an antibody cross-reactive with both IL-17A and IL-17F. In one

embodiment, the therapeutic agent is a bispecific antibody capable of binding both IL-17A and IL-17F. In one embodiment, the therapeutic agent is an anti-IL-17A/F antibody.

[0020] The present invention provides a kit for diagnosing an asthma subtype in a patient comprising (1) one or more nucleic acid molecules that hybridize with a gene, wherein the gene is selected from the group of consisting of POSTN, CST1, CST2, CCL26, CLCA1, PRR4, PRB4, SERPINB2, CEACAM5, iNOS, SERPINB4, CST4, and SERPINB10 and (2) instructions for measuring the expression levels of the gene from an asthma patient sample, wherein the elevated expression levels of any one, combination or all of said genes is indicative of the asthma subtype. According to one embodiment, the kit further comprises a gene selected from the group consisting of: PRB4, TPSD1, TPSG1, MFSD2, CPA3, GPR105, CDH26, GSN, C2ORF32, TRACH2000196 (TMEM71), DNAJC12, RGS13, SLC18A2, SH3RF2, FCER1B, RUNX2, PTGS1, and ALOX15. In one further embodiment, the gene expression level is measured by assaying for mRNA levels. In another further embodiment, the assay comprises a PCR method or the use of a microarray chip. In yet a further embodiment, the PCR method is qPCR. In one embodiment, the mRNA levels of the gene of interest relative to a control gene mRNA level greater than 2.5 fold is indicative of the asthma subtype.

[0021] The invention provides a kit for diagnosing an asthma subtype in a patient comprising (1) one or more protein molecules that bind to a protein selected from the group of consisting of POSTN, CST1, CST2, CCL26, CLCA1, PRR4, PRB4, SERPINB2, CEACAM5, iNOS, SERPINB4, CST4, and SERPINB10 and (2) instructions for measuring the expression levels of the protein from a patient sample, wherein the elevated expression levels of any one, combination or all of said proteins is indicative of the asthma subtype. In one embodiment, the kit further comprises a protein molecule that binds to a protein selected from the group consisting of: PRB4, TPSD1, TPSG1, MFSD2, CPA3, GPR105, CDH26, GSN, C2ORF32, TRACH2000196 (TMEM71), DNAJC12, RGS13, SLC18A2, SH3RF2, FCER1B, RUNX2, PTGS1, and ALOX15. In one embodiment the protein molecule is an antibody, a peptide or a peptidobody. In a further embodiment, the kit comprises a microarray chip comprising the protein molecule(s).

[0022] The present invention provides a kit for diagnosing an asthma subtype in a patient comprising instructions for measuring any one of the biomarkers from a patient sample selected from the group consisting of: serum total IgE levels, serum CEA levels, serum periostin levels, peripheral blood eosinophils and bronchoalveolar lavage (BAL) eosinophils,

wherein elevated levels of CEA, serum periostin, peripheral blood eosinophils and bronchoalveolar lavage (BAL) eosinophils. According to one embodiment, the kit provides instructions wherein an IgE level greater than 100IU/ml is indicative of the asthma subtype. According to another embodiment, the kit provides instruction, wherein a peripheral blood eosinophil level greater than $0.14 \times 10^9/L$ is indicative of the asthma subtype.

[0023] The present invention provides a kit for diagnosing an asthma subtype in a patient comprising instructions for measuring the ratio of Muc5AC:MUC5B mRNA or protein from a sample of an asthma patient, wherein a ratio greater than 25 is indicative of the asthma subtype. In one embodiment, the sample is obtained from an epithelial brushing. In another embodiment, the sample comprises airway epithelial cells. In one embodiment, the kit provides a nucleic acid molecule that hybridizes under stringent conditions with Muc5AC and a nucleic acid molecule that hybridizes under stringent conditions with MUC5B. In one embodiment, the kit provides a protein molecule that binds to Muc5AC and a protein molecule that binds to MUC5B. In one embodiment, the protein molecule is an antibody.

BRIEF DESCRIPTION OF THE DRAWINGS

[0024] Figure 1 shows gene expression levels in airway epithelium as described in Examples 1 and 2. (A) Relative expression levels of periostin (left panel), CLCA1 (middle panel), and serpinB2 (right panel) in healthy controls (N=27) and in asthmatics (N= 42) are shown. Normalized fluorescence units are indicated on the left axis of each plot. (B) Two-way comparisons of expression levels of periostin and CLCA1 (left panel), periostin and serpinB2 (middle panel), and CLCA1 and serpinB2 (right panel) in 42 asthmatics are shown. Spearman's rank order correlation (ρ) and p-values are indicated in each panel. (C) Gene expression microarray analysis for healthy controls and asthmatics identifying expression levels of periostin and co-regulated genes; IL-4/13 signature high cluster (cluster 1); IL-4/13 signature low cluster (cluster 2); healthy controls. (D) Heatmap depicting unsupervised hierarchical clustering (Euclidean complete) of periostin, CLCA1, and serpinB2 expression levels in bronchial epithelium across all subjects at baseline. (E) Mean (\pm SEM) expression levels of IL-4, IL-5, and IL-13 in bronchial biopsy homogenates obtained contemporaneously with bronchial brushings from a subset of subjects depicted in Figures 1A-D (cluster 1: 18 "IL-13 high" asthmatics; cluster 2: 16 healthy controls and 14 "IL-13 low" asthmatics). Two-way correlations across all subjects between IL-4, IL-5, and IL-13 indicated at right (Spearman's rank order correlation, ρ , and p-values).

[0025] Figure 2 shows gene families for serpins, cystatins, and PRRs, and expression levels of those genes as described in Example 3. (A) Serpins (top), cystatins (middle), and PRRs (bottom) genomic loci and organization as viewed at the University of California Santa Cruz genome browser available at <http://genome.ucsc.edu>. (B) Hierarchical clustering of all probes encoding cystatin and serpin genes as depicted in panel A. (C) Relative gene expression levels in airway epithelium of PRR4 (left panel), PRB4 (middle panel), and CEACAM5 (right panel) in healthy controls (N=27) and in asthmatics (N= 42) are shown. Normalized fluorescence units are indicated on the left axis of each plot.

[0026] Figure 3 shows microarray analysis of bronchial epithelial brushings at baseline and after one week of inhaled fluticasone propionate (ICS) treatment as described in Example 6. (A) Periostin expression; (B) PRR4 expression; (C) RUNX2 expression.

[0027] Figure 4 shows a composite graph of serum IgE and peripheral blood eosinophils in asthmatic patients as described in Examples 7 and 9.

[0028] Figure 5 shows various clinical features of IL-13 high and IL-13 low subphenotypes of asthma as described in Example 8. (A) Volume of air exhaled in the first second of a forced expiration (FEV_1), a measure of airway obstruction. (B) Improvement in FEV_1 after 4 puffs (360 μ g) of albuterol (bronchodilator reversibility testing). (C) Provocative concentration of methacholine required to induce a 20% decline in FEV_1 (PC_{20}), a measure of airway hyper-reponsiveness.

[0029] Figure 6 shows various markers of allergy, eosinophilic inflammation and airway remodeling of IL-13-high and IL-13 low subphenotypes of asthma as described in Example 8. (A) Allergen skin prick test (SPT) results using a panel of 12 aeroallergens. (B) Serum IgE concentration. (C) Peripheral blood eosinophil count. (D) Eosinophils as a percentage of total bronchoalveolar lavage fluid (BAL) cells. (E) Stereologic measurement of reticular basement membrane (RBM) thickness on endobronchial biopsy, a measure of sub-epithelial fibrosis. (F) Ratio of MUC5AC to MUC5B expression in epithelial brushings as determined by qPCR.

[0030] Figure 7 shows various clinical features of IL-13 high and IL-13 low subphenotypes of asthma as described in Example 8. (A) Percentage of subjects responding to specific aeroallergens as indicated along the bottom axis. "IL-13 low" asthma subphenotype; "IL-13 high" asthma subphenotype (*, $p<0.05$). (B) Number of positive SPT reactions vs. BAL eosinophil percentage; IL-13 asthma subphenotype as indicated (high, open squares; low, closed circles). (C) Number of positive SPT reactions vs. serum IgE; IL-13 asthma subphenotype as indicated (high, open squares; low, closed circles). (D) Number

of positive SPT reactions vs. peripheral blood eosinophil count; IL-13 asthma subphenotype as indicated (high, open squares; low, closed circles). Spearman's rank order correlation (ρ) and p-values are indicated in each plot for B-D.

[0031] Figure 8 shows airway epithelial mucin content and composition in subjects with IL-13 high and IL-13 low asthma subphenotypes and healthy controls as described in Example 8. (A) Volume of mucin per volume of epithelium, a measure of airway epithelial mucin content. (B) Expression of mucin MUC2 as determined by qPCR. (C) Expression of mucin MUC5AC as determined by qPCR. (D) Expression of mucin MUC5B as determined by qPCR.

[0032] Figure 9 shows responses of subjects with IL-13 high and IL-13 low asthma subphenotypes to inhaled corticosteroids. (A) FEV₁ measured at baseline (week 0), after 4 and 8 weeks on daily fluticasone, and one week after the cessation of fluticasone (week 9). (*): see Table 5 for number of subjects in each group and p-values. (B) Heatmap depicting unsupervised hierarchical clustering of periostin, CLCA1, and serpinB2 (as in Fig. 1D) in bronchial epithelium of asthmatics one week after the initiation of either fluticasone (N=19) or placebo treatment (N=13). Cluster identification at baseline for individual subjects and treatment are indicated below heatmap. (cluster 1: "IL-13 high" asthmatics; cluster 2: "IL-13 low" asthmatics).

[0033] Figure 10 shows alveolar macrophage gene expression in subjects with IL-13 high and IL-13 low subphenotypes of asthma as described in Example 8. Healthy controls (N=15); IL-13 low subphenotype of asthma (N=5); IL-13 high subphenotype of asthma (N=9) are indicated. The figure shows the mean (+SEM) expression levels of 15-lipoxygenase (ALOX15) and tumor necrosis factor- α (TNF- α) as determined by qPCR. (*): $p < 0.03$.

[0034] Figure 11 shows gene expression microarray analysis using 35 probes covering 28 genes of samples from healthy controls and asthmatics as described in Example 9.

[0035] Figure 12 shows gene expression microarray analysis and qPCR analysis for periostin and CEACAM5 as described in Example 9. (A) Periostin expression in healthy controls, cluster 2 asthmatics ("IL-13 LOW"), and cluster 1 asthmatics ("IL-13 high"); (B) CEACAM5 expression in healthy controls, cluster 2 asthmatics ("IL-13 LOW"), and cluster 1 asthmatics ("IL-13 HIGH"); (C) a composite graph of CEACAM5 and periostin in "IL-13 high" asthmatics (squares) and "IL-13 low" asthmatics (circles); (D) Receiver operating characteristic (ROC) analysis of an optimized algorithm for qPCR-based expression levels of

periostin and CEACAM5 showing sensitivity and specificity for healthy controls, "IL-13 high" asthmatics, and "IL-13 low" asthmatics.

[0036] Figure 13 shows serum levels of serum proteins in asthmatics and in healthy controls as described in Example 9. (A) serum levels of IgE; (B) serum levels of periostin; (C) serum levels of CEA; (D) serum levels of YKL-40; (E) serum levels of IgE in asthmatics treated with inhaled corticosteroids (ICS) (+) or not (-); (F) serum levels of periostin in asthmatics treated with inhaled corticosteroids (ICS) (+) or not (-); (G) serum levels of CEA in asthmatics treated with inhaled corticosteroids (ICS) (+) or not (-); (H) serum levels of YKL-40 in asthmatics treated with inhaled corticosteroids (ICS) (+) or not (-); (I) composite graph of serum levels of periostin in asthmatics having < 100 IU/ml serum IgE (<100) and asthmatics having ≥ 100 IU/ml serum IgE (≥ 100); (J) composite graph of serum levels of CEA in asthmatics having < 100 IU/ml serum IgE (<100) and asthmatics having ≥ 100 IU/ml serum IgE (≥ 100); (K) composite graph of serum levels of YKL-40 in asthmatics having < 100 IU/ml serum IgE (<100) and asthmatics having ≥ 100 IU/ml serum IgE (≥ 100); (L) composite graph of serum levels of periostin and CEA in asthmatics having < 100 IU/ml serum IgE (circles) and asthmatics having ≥ 100 IU/ml serum IgE (squares).

DETAILED DESCRIPTION

DEFINITIONS

[0037] Unless otherwise defined, all terms of art, notations and other scientific terminology used herein are intended to have the meanings commonly understood by those of skill in the art to which this invention pertains. In some cases, terms with commonly understood meanings are defined herein for clarity and/or for ready reference, and the inclusion of such definitions herein should not necessarily be construed to represent a substantial difference over what is generally understood in the art. The techniques and procedures described or referenced herein are generally well understood and commonly employed using conventional methodology by those skilled in the art, such as, for example, the widely utilized molecular cloning methodologies described in Sambrook et al., *Molecular Cloning: A Laboratory Manual* 2nd. edition (1989) Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. As appropriate, procedures involving the use of commercially available kits and reagents are generally carried out in accordance with manufacturer defined protocols and/or parameters unless otherwise noted.

[0038] “IL-4/IL-13 gene signature,” “IL-4/IL-13 signature,” “IL-13 gene signature,” and “IL-13 signature” are used interchangeably herein and refer to a combination of 30 genes as set forth in Table 4, or a subcombination of these 30 genes as set forth in Table 9, the gene expression pattern of which correlates with certain asthma patients. The 30 genes include POSTN, CST1, CCL26, CLCA1, CST2, PRR4, SERPINB2, CEACAM5, iNOS, SERPINB4, CST4, PRB4, TPSD1, TPSG1, MFSD2, CPA3, GPR105, CDH26, GSN, C2ORF32, TRACH2000196 (TMEM71), DNAJC12, RGS13, SLC18A2, SERPINB10, SH3RF2, FCER1B, RUNX2, PTGS1, ALOX15. The polypeptides of the IL-4/IL13 gene signature are “targeted polypeptides” of this invention.

[0039] The term “targeted polypeptide” when used herein refers to “native sequence” polypeptides and variants (which are further defined herein).

[0040] A “native sequence” polypeptide comprises a polypeptide having the same amino acid sequence as the corresponding polypeptide derived from nature. Thus, the term “native sequence polypeptide” includes naturally-occurring truncated, augmented, and frameshifted forms of a polypeptide, including but not limited to alternatively spliced forms, isoforms and polymorphisms.

[0041] “Naturally occurring variant” means a polypeptide having at least about 60% amino acid sequence identity with a reference polypeptide and retains at least one biological activity of the naturally occurring reference polypeptide. Naturally occurring variants can include variant polypeptides having at least about 65% amino acid sequence identity, at least about 70% amino acid sequence identity, at least about 75% amino acid sequence identity, at least about 80% amino acid sequence identity, at least about 80% amino acid sequence identity, at least about 85% amino acid sequence identity, at least about 90% amino acid sequence identity, at least about 95% amino acid sequence identity, at least about 98% amino acid sequence identity or at least about 99% amino acid sequence identity to a reference polypeptide.

[0042] Examples of POSTN include a polypeptide comprising SEQ ID NO:1 and other POSTN native sequence polypeptides, such as naturally occurring variants and native sequence polypeptides encoded by a nucleic acid sequence that can hybridize under stringent conditions to SEQ ID NOs:31 and/or 32.

[0043] Examples of CST1 include a polypeptide comprising SEQ ID NO:2 and other CST1 native sequence polypeptides, such as naturally occurring variants and native sequence

polypeptides encoded by a nucleic acid sequence that can hybridize under stringent conditions to SEQ ID NO:33.

[0044] Examples of CCL26 include a polypeptide comprising SEQ ID NO:3 and other CCL26 native sequence polypeptides, such as naturally occurring variants and native sequence polypeptides encoded by a nucleic acid sequence that can hybridize under stringent conditions to SEQ ID NO:34.

[0045] Examples of CLCA1 include a polypeptide comprising SEQ ID NO:4 and other CLCA1 native sequence polypeptides, such as naturally occurring variants and native sequence polypeptides encoded by a nucleic acid sequence that can hybridize under stringent conditions to SEQ ID NO:35.

[0046] Examples of CST2 include a polypeptide comprising SEQ ID NO:5 and other CST native sequence polypeptides, such as naturally occurring variants and native sequence polypeptides encoded by a nucleic acid sequence that can hybridize under stringent conditions to SEQ ID NO:36.

[0047] Examples of PRR4 include a polypeptide comprising SEQ ID NO:6 and other PRR4 native sequence polypeptides, such as naturally occurring variants and native sequence polypeptides encoded by a nucleic acid sequence that can hybridize under stringent conditions to SEQ ID NO:37.

[0048] Examples of SERPINB2 include a polypeptide comprising SEQ ID NO:7 and other SERPINB2 native sequence polypeptides, such as naturally occurring variants and native sequence polypeptides encoded by a nucleic acid sequence that can hybridize under stringent conditions to SEQ ID NO:38.

[0049] Examples of CEACAM5 include a polypeptide comprising SEQ ID NO:8 and other CEACAM5 native sequence polypeptides, such as naturally occurring variants and native sequence polypeptides encoded by a nucleic acid sequence that can hybridize under stringent conditions to SEQ ID NO:39.

[0050] Examples of iNOS include a polypeptide comprising SEQ ID NO:9 and other iNOS native sequence polypeptides, such as naturally occurring variants and native sequence polypeptides encoded by a nucleic acid sequence that can hybridize under stringent conditions to SEQ ID NO:40.

[0051] Examples of SERPINB4 include a polypeptide comprising SEQ ID NO:10 and other SERPINB4 native sequence polypeptides, such as naturally occurring variants and

native sequence polypeptides encoded by a nucleic acid sequence that can hybridize under stringent conditions to SEQ ID NOs:41 and/or 42.

[0052] Examples of CST4 include a polypeptide comprising SEQ ID NO:11 and other CST4 native sequence polypeptides, such as naturally occurring variants and native sequence polypeptides encoded by a nucleic acid sequence that can hybridize under stringent conditions to SEQ ID NO:43.

[0053] Examples of PRB4 include a polypeptide comprising SEQ ID NO:12 and other PRB4 native sequence polypeptides, such as naturally occurring variants and native sequence polypeptides encoded by a nucleic acid sequence that can hybridize under stringent conditions to SEQ ID NO:44.

[0054] Examples of TPSD1 include a polypeptide comprising SEQ ID NO:13 and other TPSD1 native sequence polypeptides, such as naturally occurring variants and native sequence polypeptides encoded by a nucleic acid sequence that can hybridize under stringent conditions to a sequence selected from the group consisting of SEQ ID NO:45-51.

[0055] Examples of TPSG1 include a polypeptide comprising SEQ ID NO:14 and other TPSG1 native sequence polypeptides, such as naturally occurring variants and native sequence polypeptides encoded by a nucleic acid sequence that can hybridize under stringent conditions a sequence selected from the group consisting of SEQ ID NO:52-55.

[0056] Examples of MFSD2 include a polypeptide comprising SEQ ID NO:15 and other MFSD2 native sequence polypeptides, such as naturally occurring variants and native sequence polypeptides encoded by a nucleic acid sequence that can hybridize under stringent conditions to SEQ ID NO:56.

[0057] Examples of CPA3 include a polypeptide comprising SEQ ID NO:16 and other CPA3 native sequence polypeptides, such as naturally occurring variants and native sequence polypeptides encoded by a nucleic acid sequence that can hybridize under stringent conditions to SEQ ID NO:57.

[0058] Examples of GPR105 include a polypeptide comprising SEQ ID NO:17 and other GPR105 native sequence polypeptides, such as naturally occurring variants and native sequence polypeptides encoded by a nucleic acid sequence that can hybridize under stringent conditions to SEQ ID NO:58.

[0059] Examples of CDH26 include a polypeptide comprising SEQ ID NO:18 and other CDH26 native sequence polypeptides, such as naturally occurring variants and native

sequence polypeptides encoded by a nucleic acid sequence that can hybridize under stringent conditions to SEQ ID NO:59.

[0060] Examples of GSN include a polypeptide comprising SEQ ID NO:19 and other GSN native sequence polypeptides, such as naturally occurring variants and native sequence polypeptides encoded by a nucleic acid sequence that can hybridize under stringent conditions to SEQ ID NO:60.

[0061] Examples of C2ORF32 include a polypeptide comprising SEQ ID NO:20 and other C2ORF32 native sequence polypeptides, such as naturally occurring variants and native sequence polypeptides encoded by a nucleic acid sequence that can hybridize under stringent conditions to SEQ ID NO:61.

[0062] Examples of TRACH2000196 (TMEM71) include a polypeptide comprising SEQ ID NO:21 and other TRACH2000196 (TMEM71) native sequence polypeptides, such as naturally occurring variants and native sequence polypeptides encoded by a nucleic acid sequence that can hybridize under stringent conditions to SEQ ID NO:62.

[0063] Examples of DNAJC12 include a polypeptide comprising SEQ ID NO:22 and other DNAJC12 native sequence polypeptides, such as naturally occurring variants and native sequence polypeptides encoded by a nucleic acid sequence that can hybridize under stringent conditions to SEQ ID NO:63.

[0064] Examples of RGS13 include a polypeptide comprising SEQ ID NO:23 and other RGS13 native sequence polypeptides, such as naturally occurring variants and native sequence polypeptides encoded by a nucleic acid sequence that can hybridize under stringent conditions to SEQ ID NO:64.

[0065] Examples of SLC18A2 include a polypeptide comprising SEQ ID NO:24 and other SLC18A2 native sequence polypeptides, such as naturally occurring variants and native sequence polypeptides encoded by a nucleic acid sequence that can hybridize under stringent conditions to SEQ ID NO:65.

[0066] Examples of SERPINB10 include a polypeptide comprising SEQ ID NO:25 and other SERPINB10 native sequence polypeptides, such as naturally occurring variants and native sequence polypeptides encoded by a nucleic acid sequence that can hybridize under stringent conditions to SEQ ID NO:66.

[0067] Examples of SH3RF2 include a polypeptide comprising SEQ ID NO:26 and other SH3RF2 native sequence polypeptides, such as naturally occurring variants and native

sequence polypeptides encoded by a nucleic acid sequence that can hybridize under stringent conditions to SEQ ID NO:67.

[0068] Examples of FCER1B include a polypeptide comprising SEQ ID NO:27 and other FCER1B native sequence polypeptides, such as naturally occurring variants and native sequence polypeptides encoded by a nucleic acid sequence that can hybridize under stringent conditions to SEQ ID NO:68.

[0069] Examples of RUNX2 include a polypeptide comprising SEQ ID NO:28 and other RUNX2 native sequence polypeptides, such as naturally occurring variants and native sequence polypeptides encoded by a nucleic acid sequence that can hybridize under stringent conditions to SEQ ID NO:69.

[0070] Examples of PTGS1 include a polypeptide comprising SEQ ID NO:29 and other PTGS1 native sequence polypeptides, such as naturally occurring variants and native sequence polypeptides encoded by a nucleic acid sequence that can hybridize under stringent conditions to SEQ ID NO:70.

[0071] Examples of ALOX15 include a polypeptide comprising SEQ ID NO:30 and other ALOX15 native sequence polypeptides, such as naturally occurring variants and native sequence polypeptides encoded by a nucleic acid sequence that can hybridize under stringent conditions to SEQ ID NO:71.

[0072] “An anti-IL13/IL4 pathway inhibitor” refers to an agent that blocks the IL-13 and/or IL-4 signalling. Examples of an anti-IL13, anti-IL4 or anti-IL13/IL4 inhibitors include, but are not limited to, anti-IL13 binding agents, anti-IL4 binding agents, anti-IL4receptoralpha binding agents, anti-IL13receptoralpha1 binding agents and anti-IL13 receptoralpha2 binding agents. Single domain antibodies that can bind IL-13, IL-4, IL-13Ralpha1, IL-13Ralpha2 or IL-4Ralpha are specifically included as inhibitors. It should be understood that molecules that can bind more than one target are included.

[0073] “Anti-IL4 binding agents” refers to agent that specifically binds to human IL-4. Such binding agents can include a small molecule, an aptamer or a polypeptide. Such polypeptide can include, but is not limited to, a polypeptide(s) selected from the group consisting of an immunoadhesin, an antibody, a peptibody and a peptide. According to one embodiment, the binding agent binds to a human IL-4 sequence with an affinity between 1 μ M – 1 pM. Specific examples of anti-IL4 binding agents can include soluble IL4Receptor alpha (e.g., extracellular domain of IL4Receptor fused to a human Fc region), anti-IL4

antibody, and soluble IL13receptoralpha1 (e.g., extracellular domain of IL13receptoralpha1 fused to a human Fc region).

[0074] “Anti-IL4receptoralpha binding agents” refers to an agent that specifically binds to human IL4 receptoralpha. Such binding agents can include a small molecule, an aptamer or a polypeptide. Such polypeptide can include, but is not limited to, a polypeptide(s) selected from the group consisting of an immunoadhesin, an antibody, a peptibody and a peptide. According to one embodiment, the binding agent binds to a human IL-4 receptor alpha sequence with an affinity between 1 μ M – 1 pM. Specific examples of anti-IL4 receptoralpha binding agents can include anti-IL4 receptor alpha antibodies.

[0075] “Anti-IL13 binding agent” refers to agent that specifically binds to human IL-13. Such binding agents can include a small molecule, aptamer or a polypeptide. Such polypeptide can include, but is not limited to, a polypeptide(s) selected from the group consisting of an immunoadhesin, an antibody, a peptibody and a peptide. According to one embodiment, the binding agent binds to a human IL-13 sequence with an affinity between 1 μ M – 1 pM. Specific examples of anti-IL13 binding agents can include anti-IL13 antibodies, soluble IL13receptoralpha2 fused to a human Fc, soluble IL4receptoralpha fused to a human Fc, soluble IL13 receptoralpha fused to a human Fc. According to one embodiment, the anti-IL13 antibody comprises the variable domains of the TNX-650 antibody (WO2005/062972). The variable domains of the TNX-650 antibody comprise (1) a VH comprising QVTLRESGPALVKPTQTLTLTCTVSGFSLSAYSVNWIRQPPGKALEWLA MIWGDGKI VYNSALKSRLTISKDTSKNQVVLTMNMDPVDATATYYCAGDGYYPYAMDNWGQG SLVTVSS (SEQ ID NO:193) and (2) a VL comprising: DIVMTQSPDLSVSLGERATINCRASKSVDSYGNFSFMHWYQQKPGQPPELLIYLASN LESGVPDRFSGSGSGTDFTLTISSLAEDVAVYYCQQNNEDPRTFGGGTKVEIK (SEQ ID NO:194). Other examples of anti-IL13 antibodies are described in WO2008/083695 (e.g., IMA-638 and IMA-026), US2008/0267959, US2008/0044420 and US2008/0248048.

[0076] “Anti-IL13receptoralpha1 binding agents” refers to an agent that specifically binds to human IL13 receptoralpha1. Such binding agents can include a small molecule, aptamer or a polypeptide. Such polypeptide can include, but is not limited to, a polypeptide(s) selected from the group consisting of an immunoadhesin, an antibody, a peptibody and a peptide. According to one embodiment, the binding agent binds to a human IL-13 receptor alpha1 sequence with an affinity between 1 μ M – 1 pM. Specific examples of anti-IL13 receptoralpha1 binding agents can include anti-IL13 receptor alpha1 antibodies.

[0077] “Anti-IL13receptoralpha2 binding agents” refers to an agent that specifically binds to human IL13 receptoralpha2. Such binding agents can include a small molecule, an aptamer or a polypeptide. Such polypeptide can include, but is not limited to, a polypeptide(s) selected from the group consisting of an immunoadhesin, an antibody, a peptibody and a peptide. According to one embodiment, the binding agent binds to a human IL-13 receptor alpha2 sequence with an affinity between 1 μ M – 1 pM. Specific examples of anti-IL13 receptoralpha2 binding agents can include anti-IL13 receptor alpha2 antibodies.

[0078] “Anti IgE binding agents” refers to an agent that specifically binds to human IgE. Such binding agents can include a small molecule, an aptamer or a polypeptide. Such polypeptide can include, but is not limited to, a polypeptide(s) selected from the group consisting of an immunoadhesin, an antibody, a peptibody and a peptide. According to one embodiment, the anti-IgE antibody comprises a VL sequence comprising Asp Ile Gln Leu Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Ser Val Asp Tyr Asp Gly Asp Ser Tyr Met Asn Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile Tyr Ala Ala Ser Tyr Leu Glu Ser Gly Val Pro Ser Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Ser His Glu Asp Pro Tyr Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys Arg Thr Val (SEQ ID NO:213) and a VH sequence comprising Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly Ser Leu Arg Leu Ser Cys Ala Val Ser Gly Tyr Ser Ile Thr Ser Gly Tyr Ser Trp Asn Trp Ile Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val Ala Ser Ile Thr Tyr Asp Gly Ser Thr Asn Tyr Asn Pro Ser Val Lys Gly Arg Ile Thr Ile Ser Arg Asp Asp Ser Lys Asn Thr Phe Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys Ala Arg Gly Ser His Tyr Phe Gly His Trp His Phe Ala Val Trp Gly Gln Gly (SEQ ID NO:214).

[0079] “Anti-M1’ binding agents” refers to an agent that specifically binds to the membrane proximal M1’ region of surface expressed IgE on B cells. Such binding agents can include a small molecule, an aptamer or a polypeptide. Such polypeptide can include, but is not limited to, a polypeptide(s) selected from the group consisting of an immunoadhesin, an antibody, a peptibody and a peptide. According to one embodiment, the anti-IgE antibody comprises an antibody described in WO2008/116149 or a variant thereof.

[0080] The term “small molecule” refers to an organic molecule having a molecular weight between 50 Daltons to 2500 Daltons.

[0081] The term "antibody" is used in the broadest sense and specifically covers, for example, monoclonal antibodies, polyclonal antibodies, antibodies with polypeptidic specificity, single chain antibodies, multi-specific antibodies and fragments of antibodies. Such antibodies can be chimeric, humanized, human and synthetic. Such antibodies and methods of generating them are described in more detail below.

[0082] The term "variable" refers to the fact that certain segments of the variable domains differ extensively in sequence among antibodies. The V regions mediate antigen binding and define specificity of a particular antibody for its particular antigen. However, the variability is not evenly distributed across the 110-amino acid span of the variable domains. Instead, the V domains consist of relatively invariant stretches called framework regions (FRs) of 15-30 amino acids separated by shorter regions of extreme variability called "hypervariable regions" that are each 9-12 amino acids long. The variable domains of native heavy and light chains each comprise four FRs, largely adopting a beta-sheet configuration, connected by three hypervariable regions, which form loops connecting, and in some cases forming part of, the beta-sheet structure. The hypervariable regions in each chain are held together in close proximity by the FRs and, with the hypervariable regions from the other chain, contribute to the formation of the antigen-binding site of antibodies (see Kabat et al., Sequences of Proteins of Immunological Interest, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, MD. (1991)). The constant domains are not involved directly in binding an antibody to an antigen, but exhibit various effector functions, such as participation of the antibody in antibody dependent cellular cytotoxicity (ADCC).

[0083] The term "hypervariable region" (or "HVR") when used herein refers to the amino acid residues of an antibody which are responsible for antigen-binding. The hypervariable region generally comprises amino acid residues from a "complementarity determining region" or "CDR" (e.g. around about residues 24-34 (L1), 50-56 (L2) and 89-97 (L3) in the VL, and around about 31-35B (H1), 50-65 (H2) and 95-102 (H3) in the VH (Kabat et al., Sequences of Proteins of Immunological Interest, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, MD. (1991)) and/or those residues from a "hypervariable loop" (e.g. residues 26-32 (L1), 50-52 (L2) and 91-96 (L3) in the VL, and 26-32 (H1), 52A-55 (H2) and 96-101 (H3) in the VH (Chothia and Lesk J. Mol. Biol. 196:901-917 (1987)).

[0084] Hypervariable regions may comprise "extended hypervariable regions" as follows: 24-36 (L1), 46-56 (L2) and 89-97 (L3) in the VL and 26-35B (H1), 47-65 (H2) and 93-102

(H3) in the VH. The variable domain residues are numbered according to Kabat et al., *supra* for each of these definitions.

[0085] "Framework" or "FR" residues are those variable domain residues other than the hypervariable region residues as herein defined. For example, light chain framework 1 (LC-FR1), framework 2 (LC-FR2), framework 3 (LC-FR3) and framework 4 (LC-FR4) region may comprise residues numbered 1-23, 35-49, 57-88 and 98-107 of an antibody (Kabat numbering system), respectively. In another example, heavy chain framework 1 (HC-FR1), heavy chain framework 2 (HC-FR2), heavy chain framework 3 (HC-FR3) and heavy chain framework 4 (HC-FR4) may comprise residues 1-25, 36-48, 66-92 and 103-113, respectively, of an antibody (Kabat numbering system).

[0086] As referred to herein, the "consensus sequence" or consensus V domain sequence is an artificial sequence derived from a comparison of the amino acid sequences of known human immunoglobulin variable region sequences.

[0087] The term "monoclonal antibody" as used herein refers to an antibody from a population of substantially homogeneous antibodies, *i.e.*, the individual antibodies comprising the population are identical and/or bind the same epitope(s), except for possible variants that may arise during production of the monoclonal antibody, such variants generally being present in minor amounts. Such monoclonal antibody typically includes an antibody comprising a polypeptide sequence that binds a target, wherein the target-binding polypeptide sequence was obtained by a process that includes the selection of a single target binding polypeptide sequence from a plurality of polypeptide sequences. For example, the selection process can be the selection of a unique clone from a plurality of clones, such as a pool of hybridoma clones, phage clones or recombinant DNA clones. It should be understood that the selected target binding sequence can be further altered, for example, to improve affinity for the target, to humanize the target binding sequence, to improve its production in cell culture, to reduce its immunogenicity *in vivo*, to create a multispecific antibody, *etc.*, and that an antibody comprising the altered target binding sequence is also a monoclonal antibody of this invention. In contrast to polyclonal antibody preparations which typically include different antibodies directed against different determinants (epitopes), each monoclonal antibody of a monoclonal antibody preparation is directed against a single determinant on an antigen. In addition to their specificity, the monoclonal antibody preparations are advantageous in that they are typically uncontaminated by other immunoglobulins. The modifier "monoclonal" indicates the character of the antibody as being obtained from a substantially homogeneous

population of antibodies, and is not to be construed as requiring production of the antibody by any particular method. For example, the monoclonal antibodies to be used in accordance with the present invention may be made by a variety of techniques, including the hybridoma method (e.g., Kohler *et al.*, *Nature*, 256:495 (1975); Harlow *et al.*, *Antibodies: A Laboratory Manual*, (Cold Spring Harbor Laboratory Press, 2nd ed. 1988); Hammerling *et al.*, in: *Monoclonal Antibodies and T-Cell Hybridomas* 563-681, (Elsevier, N.Y., 1981), recombinant DNA methods (see, e.g., U.S. Patent No. 4,816,567), phage display technologies (see, e.g., Clackson *et al.*, *Nature*, 352:624-628 (1991); Marks *et al.*, *J. Mol. Biol.*, 222:581-597 (1991); Sidhu *et al.*, *J. Mol. Biol.* 338(2):299-310 (2004); Lee *et al.*, *J. Mol. Biol.* 340(5):1073-1093 (2004); Fellouse, *Proc. Nat. Acad. Sci. USA* 101(34):12467-12472 (2004); and Lee *et al.* *J. Immunol. Methods* 284(1-2):119-132 (2004) and technologies for producing human or human-like antibodies from animals that have parts or all of the human immunoglobulin loci or genes encoding human immunoglobulin sequences (see, e.g., WO98/24893, WO/9634096, WO/9633735, and WO/91 10741, Jakobovits *et al.*, *Proc. Natl. Acad. Sci. USA*, 90:2551 (1993); Jakobovits *et al.*, *Nature*, 362:255-258 (1993); Bruggemann *et al.*, *Year in Immunol.*, 7:33 (1993); U.S. Patent Nos. 5,545,806, 5,569,825, 5,591,669 (all of GenPharm); 5,545,807; WO 97/17852, U.S. Patent Nos. 5,545,807; 5,545,806; 5,569,825; 5,625,126; 5,633,425; and 5,661,016, and Marks *et al.*, *Bio/Technology*, 10: 779-783 (1992); Lonberg *et al.*, *Nature*, 368: 856-859 (1994); Morrison, *Nature*, 368: 812-813 (1994); Fishwild *et al.*, *Nature Biotechnology*, 14: 845-851 (1996); Neuberger, *Nature Biotechnology*, 14: 826 (1996); and Lonberg and Huszar, *Intern. Rev. Immunol.*, 13: 65-93 (1995).

[0088] The monoclonal antibodies herein specifically include "chimeric" antibodies (immunoglobulins) in which a portion of the heavy and/or light chain is identical with or homologous to corresponding sequences in antibodies derived from a particular species or belonging to a particular antibody class or subclass, while portions of the remainder of the chain(s) is identical with or homologous to corresponding sequences in antibodies derived from another species or belonging to another antibody class or subclass, as well as fragments of such antibodies, so long as they exhibit the desired biological activity (U.S. Patent No. 4,816,567; Morrison *et al.*, *Proc. Natl. Acad. Sci. USA*, 81:6851-6855 (1984)). Methods of making chimeric antibodies are known in the art.

[0089] "Humanized" forms of non-human (e.g., murine) antibodies are chimeric immunoglobulins, immunoglobulin chains or fragments thereof (such as Fv, Fab, Fab', F(ab')₂ or other antigen-binding subsequences of antibodies) which contain minimal

sequence derived from non-human immunoglobulin. In some embodiments, humanized antibodies are human immunoglobulins (recipient antibody) in which residues from a complementarity-determining region (CDR) of the recipient are replaced by residues from a CDR of a non-human species (donor antibody) such as mouse, rat or rabbit having the desired specificity, affinity, and capacity. In some instances, Fv framework region (FR) residues of the human immunoglobulin are replaced by corresponding non-human residues. Furthermore, humanized antibodies may comprise residues which are found neither in the recipient antibody nor in the imported CDR or framework sequences. These modifications are generally made to further refine and maximize antibody performance. Typically, the humanized antibody will comprise substantially all of at least one variable domain, in which all or substantially all of the hypervariable loops derived from a non-human immunoglobulin and all or substantially all of the FR regions are derived from a human immunoglobulin sequence although the FR regions may include one or more amino acid substitutions to, e.g., improve binding affinity. In one preferred embodiment, the humanized antibody will also comprise at least a portion of an immunoglobulin constant region (Fc), typically that of a human immunoglobulin or a human consensus constant sequence. For further details, see Jones et al., *Nature*, 321:522-525 (1986); Reichmann et al., *Nature*, 332:323-329 (1988); and Presta, *Curr. Op. Struct. Biol.*, 2:593-596 (1992). The humanized antibody includes a PRIMATIZED[®] antibody wherein the antigen-binding region of the antibody is derived from an antibody produced by, e.g., immunizing macaque monkeys with the antigen of interest. Methods of making humanized antibodies are known in the art.

[0090] Human antibodies can also be produced using various techniques known in the art, including phage-display libraries. Hoogenboom and Winter, *J. Mol. Biol.*, 227:381 (1991); Marks et al., *J. Mol. Biol.*, 222:581 (1991). The techniques of Cole et al. and Boerner et al. are also available for the preparation of human monoclonal antibodies. Cole et al., *Monoclonal Antibodies and Cancer Therapy*, Alan R. Liss, p. 77 (1985); Boerner et al., *J. Immunol.*, 147(1):86-95 (1991). See also, Lonberg and Huszar, *Int. Rev. Immunol.* 13:65-93 (1995). PCT publications WO 98/24893; WO 92/01047; WO 96/34096; WO 96/33735; European Patent No. 0 598 877; U.S. Pat. Nos. 5,413,923; 5,625,126; 5,633,425; 5,569,825; 5,661,016; 5,545,806; 5,814,318; 5,885,793; 5,916,771; and 5,939,598.

[0091] "Antibody fragments" comprise a portion of a full length antibody, generally the antigen binding or variable region thereof. Examples of antibody fragments include Fab, Fab',

F(ab')₂, and Fv fragments; diabodies; linear antibodies; single-chain antibody molecules; and multispecific antibodies formed from antibody fragments.

[0092] "Fv" is the minimum antibody fragment which contains a complete antigen-recognition and -binding site. This fragment consists of a dimer of one heavy- and one light-chain variable region domain in tight, non-covalent association. From the folding of these two domains emanate six hypervariable loops (3 loops each from the H and L chain) that contribute the amino acid residues for antigen binding and confer antigen binding specificity to the antibody. However, even a single variable domain (or half of an Fv comprising only three CDRs specific for an antigen) may have the ability to recognize and bind antigen, although at a lower affinity than the entire binding site.

[0093] "Functional fragments" of the antibodies of the invention are those fragments that retain binding to polypeptide with substantially the same affinity as the intact full chain molecule from which they are derived and are active in at least one assay (e.g., inhibition of TH2-induced asthma pathway such as in mouse models or inhibition of a biological activity of the antigen that binds to the antibody fragment *in vitro*).

[0094] Antibody "effector functions" refer to those biological activities attributable to the Fc region (a native sequence Fc region or amino acid sequence variant Fc region) of an antibody, and vary with the antibody isotype. Examples of antibody effector functions include: C1q binding and complement dependent cytotoxicity; Fc receptor binding; antibody-dependent cell-mediated cytotoxicity (ADCC); phagocytosis; down regulation of cell surface receptors (e.g. B cell receptor); and B cell activation. A "native sequence Fc region" comprises an amino acid sequence identical to the amino acid sequence of an Fc region found in nature.

[0095] "Percent (%) amino acid sequence identity" or "homology" with respect to the polypeptide and antibody sequences identified herein is defined as the percentage of amino acid residues in a candidate sequence that are identical with the amino acid residues in the polypeptide being compared, after aligning the sequences considering any conservative substitutions as part of the sequence identity. Alignment for purposes of determining percent amino acid sequence identity can be achieved in various ways that are within the skill in the art, for instance, using publicly available computer software such as BLAST, BLAST-2, ALIGN or Megalign (DNASTAR) software. Those skilled in the art can determine appropriate parameters for measuring alignment, including any algorithms needed to achieve maximal alignment over the full length of the sequences being compared. For purposes

herein, however, % amino acid sequence identity values are generated using the sequence comparison computer program ALIGN-2. The ALIGN-2 sequence comparison computer program was authored by Genentech, Inc. and the source code has been filed with user documentation in the U.S. Copyright Office, Washington D.C., 20559, where it is registered under U.S. Copyright Registration No. TXU510087. The ALIGN-2 program is publicly available through Genentech, Inc., South San Francisco, California. The ALIGN-2 program should be compiled for use on a UNIX operating system, preferably digital UNIX V4.0D. All sequence comparison parameters are set by the ALIGN-2 program and do not vary.

[0096] The term “Fc region-comprising polypeptide” refers to a polypeptide, such as an antibody or immunoadhesin (see definitions below), which comprises an Fc region. The C-terminal lysine (residue 447 according to the EU numbering system) of the Fc region may be removed, for example, during purification of the polypeptide or by recombinantly engineering the nucleic acid encoding the polypeptide. Accordingly, a composition comprising polypeptides, including antibodies, having an Fc region according to this invention can comprise polypeptides populations with all K447 residues removed, polypeptide populations with no K447 residues removed or polypeptide populations having a mixture of polypeptides with and without the K447 residue.

[0097] Throughout the present specification and claims, the Kabat numbering system is generally used when referring to a residue in the variable domain (approximately, residues 1-107 of the light chain and residues 1-113 of the heavy chain) (e.g., Kabat et al., *Sequences of Immunological Interest*, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, Md. (1991)). The “EU numbering system” or “EU index” is generally used when referring to a residue in an immunoglobulin heavy chain constant region (e.g., the EU index reported in Kabat et al., *Sequences of Proteins of Immunological Interest*, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, MD (1991) expressly incorporated herein by reference). Unless stated otherwise herein, references to residues numbers in the variable domain of antibodies means residue numbering by the Kabat numbering system. Unless stated otherwise herein, references to residue numbers in the constant domain of antibodies means residue numbering by the EU numbering system (e.g., see United States Provisional Application No. 60/640,323, Figures for EU numbering).

[0098] “Stringency” of hybridization reactions is readily determinable by one of ordinary skill in the art, and generally is an empirical calculation dependent upon probe length, washing temperature, and salt concentration. In general, longer probes require higher

temperatures for proper annealing, while shorter probes need lower temperatures. Hybridization generally depends on the ability of denatured DNA to reanneal when complementary strands are present in an environment below their melting temperature. The higher the degree of desired homology between the probe and hybridizable sequence, the higher the relative temperature which can be used. As a result, it follows that higher relative temperatures would tend to make the reaction conditions more stringent, while lower temperatures less so. For additional details and explanation of stringency of hybridization reactions, see Ausubel et al., Current Protocols in Molecular Biology, Wiley Interscience Publishers, (1995).

[0099] "Stringent conditions" or "high stringency conditions", as defined herein, can be identified by those that: (1) employ low ionic strength and high temperature for washing, for example 0.015 M sodium chloride/0.0015 M sodium citrate/0.1% sodium dodecyl sulfate at 50°C; (2) employ during hybridization a denaturing agent, such as formamide, for example, 50% (v/v) formamide with 0.1% bovine serum albumin/0.1% Ficoll/0.1% polyvinylpyrrolidone/50mM sodium phosphate buffer at pH 6.5 with 750 mM sodium chloride, 75 mM sodium citrate at 42°C; or (3) overnight hybridization in a solution that employs 50% formamide, 5 x SSC (0.75 M NaCl, 0.075 M sodium citrate), 50 mM sodium phosphate (pH 6.8), 0.1% sodium pyrophosphate, 5 x Denhardt's solution, sonicated salmon sperm DNA (50 µg/ml), 0.1% SDS, and 10% dextran sulfate at 42°C, with a 10 minute wash at 42°C in 0.2 x SSC (sodium chloride/sodium citrate) followed by a 10 minute high-stringency wash consisting of 0.1 x SSC containing EDTA at 55°C.

[00100] "Moderately stringent conditions" can be identified as described by Sambrook et al., Molecular Cloning: A Laboratory Manual, New York: Cold Spring Harbor Press, 1989, and include the use of washing solution and hybridization conditions (e.g., temperature, ionic strength and %SDS) less stringent than those described above. An example of moderately stringent conditions is overnight incubation at 37°C in a solution comprising: 20% formamide, 5 x SSC (150 mM NaCl, 15 mM trisodium citrate), 50 mM sodium phosphate (pH 7.6), 5 x Denhardt's solution, 10% dextran sulfate, and 20 mg/ml denatured sheared salmon sperm DNA, followed by washing the filters in 1 x SSC at about 37-50°C. The skilled artisan will recognize how to adjust the temperature, ionic strength, etc. as necessary to accommodate factors such as probe length and the like.

[00101] As used herein, a subject to be treated is a mammal (e.g., human, non-human primate, rat, mouse, cow, horse, pig, sheep, goat, dog, cat, etc.). The subject may be a

clinical patient, a clinical trial volunteer, an experimental animal, etc. The subject may be suspected of having or at risk for having asthma or be diagnosed with asthma. According to one preferred embodiment, the subject to be treated according to this invention is a human.

[00102] "Treating" or "treatment" or "alleviation" refers to measures, wherein the object is to prevent or slow down (lessen) the targeted pathologic condition or disorder or relieve some of the symptoms of the disorder. Those in need of treatment include can include those already with the disorder as well as those prone to have the disorder or those in whom the disorder is to be prevented. A subject or mammal is successfully "treated" for asthma if, after receiving a therapeutic agent of the present invention, the patient shows observable and/or measurable reduction in or absence of one or more of the following: recurrent wheezing, coughing, trouble breathing, chest tightness, symptoms that occur or worsen at night, symptoms that are triggered by cold air, exercise or exposure to allergens.

[00103] The term "therapeutically effective amount" refers to an amount of a polypeptide of this invention effective to "alleviate" or "treat" a disease or disorder in a subject.

[00104] "Chronic" administration refers to administration of the agent(s) in a continuous mode as opposed to an acute mode, so as to maintain the initial therapeutic effect (activity) for an extended period of time. "Intermittent" administration is treatment that is not consecutively done without interruption, but rather is cyclic in nature.

[00105] "Forced expiratory volume (FEV1)" refers to a standard test that measures the volume of air expelled in the first second of a forced expiration. FEV1 is measured by a spirometer, which consists of a mouthpiece and disposable tubing connected to a machine that records the results and displays them on a graph. To perform spirometry, a person inhales deeply, closes the mouth tightly around the tube and then exhales through the tubing while measurements are taken. The volume of air exhaled, and the length of time each breath takes is recorded and analyzed. Spirometry results are expressed as a percentage. Examples of normal spirometry results include a FEV1 of 75 percent of vital capacity after one second. An example of abnormal spirometry results include a reading of less than 80 percent of the normal predicted value. An abnormal result usually indicates the presence of some degree of obstructive lung disease such as asthma, emphysema or chronic bronchitis, or restrictive lung disease such as pulmonary fibrosis. For example, FEV1 values (percentage of predicted) can be used to classify the obstruction that may occur with asthma and other obstructive lung diseases like emphysema or chronic bronchitis: FEV1 65 percent to 79 percent predicted =

mild obstruction, FEV1 40 percent to 59 percent predicted = moderate obstruction, and FEV1 less than 40 percent predicted = severe obstruction.

[00106] Examples of nucleic acid probes that may be used to identify the proteins described herein (e.g., by microarray analysis), include, but are not limited to the probes described in Table 4.

[00107] "Elevated expression level" or "elevated levels" refers to an increased expression of a mRNA or a protein in a patient relative to a control, such as an individual or individuals who are not suffering from asthma.

[00108] All publications (including patents and patent applications) cited herein are hereby incorporated in their entirety by reference.

[00109] Throughout this specification and claims, the word "comprise," or variations such as "comprises" or "comprising," will be understood to imply the inclusion of a stated integer or group of integers but not the exclusion of any other integer or group of integers.

[00110] The foregoing written description is considered to be sufficient to enable one skilled in the art to practice the invention. The following Examples are offered for illustrative purposes only, and are not intended to limit the scope of the present invention in any way. Indeed, various modifications of the invention in addition to those shown and described herein will become apparent to those skilled in the art from the foregoing description and fall within the scope of the appended claims.

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[00111] All references cited herein, including patent applications and publications, are incorporated by reference in their entirety for any purpose. In addition, United States Provisional Applications US Serial No. 61/072,572, filed 3/31/2008, US Serial No. 61/041,480, filed 4/1/2008, US Serial No.: 61/128,383, filed 5/20/2008, US Serial No.: 61/205,392, filed 1/16/2009 are incorporated by reference in their entirety. Also, specifically PCT publications WO2005/062972 and WO2008/116149 are incorporated by reference by their entirety.

EXAMPLES

Example 1 – Methods

Airway Tissue Bank

[00112] We studied biological samples stored in the Airway Tissue Bank at the University of California, San Francisco (UCSF) that had been collected during bronchoscopy performed for research purposes in healthy and asthmatic volunteers. Research bronchoscopy had included collection of epithelial brushings, bronchoalveolar lavage (BAL) and bronchial biopsies using specific methods previously described [8, 46]. BAL cell counts and differentials had been performed and databased, and macrophages had been sorted from BAL

fluid using flow cytometry [51]. Four to six bronchial biopsies had been obtained from 2nd-through 5th-order carinae (contralateral to the brushing site), formalin-fixed, and then paraffin-embedded in isotropic uniform random orientation [31] to enable quantitative measures of inflammation and remodeling using methods of design-based stereology [52]. An additional 2 bronchial biopsies had been homogenized and processed for RNA using the Qiagen RNeasy minikit (Qiagen Inc., Valencia, CA). RNA extracted from epithelial brushings, homogenates of bronchial biopsies, and lavage macrophages had been quality assured and aliquoted for future microarray- and PCR-based gene profiling. All research bronchoscopy studies had been approved by the UCSF Committee on Human Research (CHR), written informed consent had been obtained from all subjects, and all studies had been performed in accordance with the principles expressed in the Declaration of Helsinki. The Airway Tissue Bank procedures were also reviewed and approved by UCSF's CHR. Samples of epithelial brushings and macrophages from this tissue bank have been used in previously reported studies [8, 14, 46, 51, 53]. Most recently, microarray analyses of differentially expressed genes in epithelial brushings in asthmatic subjects have been reported by us [8].

[00113] For the purposes of identifying subsets of patients with asthma who differ with respect to the molecular mechanism underlying their airway inflammation and the distinct inflammatory, pathological and clinical phenotypes characteristic of these subsets, we first conducted new analyses on our previously generated epithelial cell microarray data, and we then supplemented these new analyses with review of additional and detailed clinical characterization data (including data on bronchodilator reversibility and allergen skin test reactivity) from these same subjects and newly generated data, including: (i) gene expression profiles in homogenates of bronchial biopsies and alveolar macrophages; (ii) quantitative measures of subepithelial collagen and airway epithelial mucin in bronchial biopsies; (iii) total and differential cell counts in BAL.

Human Subjects and Samples

[00114] Subjects with asthma (N=42) had a prior physician diagnosis of asthma, symptoms consistent with asthma confirmed by a study physician, airway hyper-responsiveness (defined as a drop in forced expiratory volume in the first second (FEV₁) of 20% or greater with inhalation of <8 mg/mL of methacholine [PC₂₀ methacholine] and *either*: 1) symptoms on 2 or more days per week, 2) β -agonist use on 2 or more days per week, or 3)

an FEV₁<85% predicted. They did not take inhaled or oral corticosteroids for 4 weeks prior to enrollment. Healthy controls (N=27) had no history of lung disease and lacked airway hyper-responsiveness (PC₂₀ methacholine >16 mg/mL). Certain studies included current smokers without asthma (N=16). Exclusion criteria for all subjects included upper respiratory tract infection in the previous 4 weeks, asthma exacerbation within 6 weeks and current use of salmeterol, astemizole, nedocromil sodium, sodium cromoglycate, methylxanthines, montelukast or zafirlukast. Subjects underwent baseline evaluation by study physicians (including spirometry and methacholine challenge testing as described previously [8]). Subjects also underwent allergen skin prick testing (ASPT) with a panel of 12 aeroallergens, a positive control and a negative control (Table 6).

[00115] Thirty-two of the subjects with asthma had also been enrolled in a double-blind randomized controlled clinical trial of inhaled fluticasone (500 µg, twice daily, N=19) or matched placebo (N=13) (ClinicalTrials.gov Identifier: NCT00187499). The trial was designed to determine the effects of inhaled steroid (fluticasone) on airway gene expression and to relate gene expression changes to improvements in lung function. The asthma subjects in the clinical trial had undergone baseline bronchoscopy and had been randomized to receive study medication before undergoing repeat bronchoscopy one week later after starting study drug. Asthma subjects continued study medication for a total of 8 weeks. Healthy control subjects and smokers were enrolled in one of three cross-sectional studies, which comprised two visits each, the first for characterization and the second for bronchoscopy 1 week later. Thirty-five subjects had adequate baseline bronchoscopy, and 32 had RNA available from epithelial brushings at both bronchoscopies. Lung function was measured (by spirometry) after 4 weeks and 8 weeks on study medication, and a final spirometry was completed after a one week run-out. Methods for bronchoscopy, epithelial brushing, bronchoalveolar lavage, spirometry, and sample handling were identical across all studies.

[00116] Bronchoalveolar lavage (BAL) was performed by instilling 4 aliquots of 50ml of sterile saline into either the lingula or right middle lobe, with recovery by suction. Cell counts were performed using a hemocytometer and Turks solution (1% glacial acetic acid and 0.01% gentian violet in distilled H₂O). Then BAL cell differentials were performed on cytocentrifuged preparations using the Shandon Kwik-Diff stain kit (Thermo Fisher Scientific, Waltham MA). Thirty-two of the subjects with asthma were also enrolled in a double-blind randomized controlled clinical trial of inhaled fluticasone (500 mcg BID) or matched placebo. In addition to the inclusion criteria above, these subjects were also required to have *either*

asthma symptoms on 2 or more days per week, or β -agonist use on 2 or more days per week, or $FEV_1 < 85\%$ predicted. Subjects in the clinical trial underwent a baseline visit and baseline bronchoscopy as described above, were randomized to receive study medication and underwent repeat bronchoscopy one week later. Then, they continued study medication for a total of 8 weeks with scheduled re-assessment of spirometry and methacholine challenge testing. All clinical studies were approved by the University of California at San Francisco Committee on Human Research, written informed consent was obtained from all subjects, and all studies were performed in accordance with the principles expressed in the Declaration of Helsinki.

Microarray Analyses and Morphometry

[00117] Microarray data from mild-moderate non-smoking asthma patients and healthy non-smoking subjects were obtained from a previous study as described [8]. Methodological detail and microarray data are also available from the Gene Expression Omnibus public database, which can be accessed online at the National Center for Biotechnology Information, accession number GSE4302. Microarray data was analysed in the present study to determine whether genes were differentially regulated within the asthmatic group. Also, the microarray data was analyzed to determine whether other genes were co-regulated with top asthma-related, IL-13 induced genes. Two step real-time PCR (qPCR) was performed as described previously [45] using the primers and probes in Table 1 (i.e., multiplex PCR followed by real time PCR on cDNA generated products).

[00118] Morphometric analyses were performed by applying design-based stereology to 4-6 endobronchial biopsies from each subject as described previously. Specifically, analysis of reticular basement membrane thickness was measured in trichrome $3\mu m$ sections using the orthogonal intercept method [31]. Airway mucin content was measured in Alcian blue/Periodic acid Schiff $3\mu m$ sections using point and line intersect counting methods [46].

Statistical Methods

[00119] Microarray preprocessing was performed using RMA with Bioconductor open source software [47] in the R statistical environment. Unsupervised hierarchical clustering was performed using the Euclidean metric with complete linkage. All other statistical analyses including were performed using the JMP statistical analysis software package (SAS Institute, Cary, NC). Values are presented as mean \pm standard deviation or median (range)

unless otherwise specified. Correlation was performed using Spearman's rank order correlation. For significance testing of PC₂₀ and serum IgE levels, data were log transformed for normality. A $p < 0.05$ was taken as statistically significant and sidak correction for multiple comparisons was employed after initial three-group comparisons by ANOVA.

Table 1. Primer and probe sequences for qPCR

Gene	Type	Sequence
IL-13	RT-forward	GGATGCTGAGCGGATTCTG [SEQ ID NO:73]
	RT-reverse	CCCTCGCGAAAAAGTTTCTT [SEQ ID NO:74]
	Taqman-forward	AAGGTCTCAGCTGGGCAGTTT [SEQ ID NO:75]
	Taqman-reverse	AAACTGGGCCACCTCGATT [SEQ ID NO:76]
	probe	CCAGCTTGATGTCCGAGACACCA [SEQ ID NO:77]
IL-4	RT-forward	GGGTCTCACCTCCCAACTGC [SEQ ID NO:78]
	RT-reverse	TGTCTGTTACGGTCAACTCGGT [SEQ ID NO:79]
	Taqman-forward	GCTTCCCCTCTGTTCTTCCT [SEQ ID NO:80]
	Taqman-reverse	GCTCTGTGAGGCTGTTCAAAGTT [SEQ ID NO:81]
	probe	TCCACGGACACAAGTGCGATATCACC [SEQ ID NO:82]
IL-5	RT-forward	GCCATGAGGATGCTTCTGCA [SEQ ID NO:83]
	RT-reverse	GAATCCTCAGAGTCTCATTGGCTATC [SEQ ID NO:84]
	Taqman-forward	AGCTGCCTACGTGTATGCCA [SEQ ID NO:85]
	Taqman-reverse	GTGCCAAGGCTCTTTTACCA [SEQ ID NO:86]
	probe	CCCCACAGAAATCCCAAGTGCA [SEQ ID NO:87]
MUC2	RT-forward	ACTCTCTACCTCCATCAATAACTCC [SEQ ID NO:88]
	RT-reverse	TGGCTCTGCAAGAGATGTTAGCT [SEQ ID NO:89]
	Taqman-forward	GCTGGCTGGATTCTGGAAAA [SEQ ID NO:90]
	Taqman-reverse	TGGCTCTGCAAGAGATGTTAGC [SEQ ID NO:91]
	probe	TCTCCAATCAATTCTGTGTCTCCACCTGG [SEQ ID NO:92]
MUC5ac2	RT-forward	TGTGGCGGGAAGACAGC [SEQ ID NO:93]
	RT-reverse	CCTTCCTATGGCTTAGCTTCAGC [SEQ ID NO:94]
	Taqman-forward	CGTGTGTGCACCGAGAACGT [SEQ ID NO:95]
	Taqman-reverse	ATCTTGATGGCCTTGGAGCA [SEQ ID NO:96]
	probe	CTGCGGCACCACAGGGACCA [SEQ ID NO:97]
MUC5b	RT-forward	TTGAGGACCCCTGCTCCCT [SEQ ID NO:98]
	RT-reverse	AGGCGTGCACATAGGAGGAC [SEQ ID NO:99]
	Taqman-forward	CGATCCCAACAGTGCCTTCT [SEQ ID NO:100]
	Taqman-reverse	CCTCGCTCCGCTCACAGT [SEQ ID NO:101]
	probe	CAACCCCAAGCCCTTCACTCGA [SEQ ID NO:102]
ALOX15	RT-forward	CCAACCACCAAGGATGCAA [SEQ ID NO:103]
	RT-reverse	TCTGCCAGCTGCCAAGT [SEQ ID NO:104]
	Taqman-forward	CCAACCACCAAGGATGCAA [SEQ ID NO:105]
	Taqman-reverse	GGAGAGAAGCCTGGTGGAAAT [SEQ ID NO:106]

	probe	CAGTGTGCGCCATCACTGTCTCCAGC [SEQ ID NO:107]
ALOX5	RT-forward	ACGTCCACCAGACCATCACC [SEQ ID NO:108]
	RT-reverse	GAATCTCACGTGTGCCACCA [SEQ ID NO:109]
	Taqman-forward	ATTGCAATGTACCGCCAGC [SEQ ID NO:110]
	Taqman-reverse	GAATCTCACGTGTGCCACCA [SEQ ID NO:111]
	probe	CTGCTGTGCACCCCATTTTCAAGCTG [SEQ ID NO:112]
ALOX5AP	RT-forward	CATAAAGTGGAGCACGAAAGCA [SEQ ID NO:113]
	RT-reverse	GGTACGCATCTACACAGTTCTGGTT [SEQ ID NO:114]
	Taqman-forward	CAGAATGGGAGGAGCTTCCA [SEQ ID NO:115]
	Taqman-reverse	CACAGTTCTGGTTGGCAGTGTAG [SEQ ID NO:116]
	probe	CCGGAACACTTGCCTTTGAGCGG [SEQ ID NO:117]
ARG1	RT-forward	CAAGGTCTGTGGGAAAAGCAA [SEQ ID NO:118]
	RT-reverse	TGGCCAGAGATGCTTCCAAT [SEQ ID NO:119]
	Taqman-forward	GCAGAAGTCAAGAAGACGGAAGA [SEQ ID NO:120]
	Taqman-reverse	TGCTTCCAATTGCCAAACTG [SEQ ID NO:121]
	probe	TCTCCGCCACGACACAGGCT [SEQ ID NO:122]
IL1B	RT-forward	ACTTAAAGCCCGCTGACAGA [SEQ ID NO:123]
	RT-reverse	GCTACTTCTTGCCCCCTTTGAA [SEQ ID NO:124]
	Taqman-forward	CCACGGCCACATTTGGTT [SEQ ID NO:125]
	Taqman-reverse	AGGGAAGCGGTTGCTCATC [SEQ ID NO:126]
	probe	AGAAACCCCTCTGTCAATTCGCTCCACAT [SEQ ID NO:127]
IL1rn	RT-forward	CTCCGCAGTCACCTAATCACTCT [SEQ ID NO:128]
	RT-reverse	GGCTCAATGGGTACCACATCTATCT [SEQ ID NO:129]
	Taqman-forward	TTCTGTTCATTACAGAGACGAT [SEQ ID NO:130]
	Taqman-reverse	AGATTCTGAAGGCTTGCATCTTG [SEQ ID NO:131]
	probe	TGCCGACCCTCTGGGAGAAAATCC [SEQ ID NO:132]
LTA4H	RT-forward	ATTCAAGGATCTTGCTGCCTTT [SEQ ID NO:133]
	RT-reverse	TGCAGTCACGGGATGCAT [SEQ ID NO:134]
	Taqman-forward	CAAGGATCTTGCTGCCTTTGA [SEQ ID NO:135]
	Taqman-reverse	TGCTTGCTTTGTGCTCTTGGT [SEQ ID NO:136]
	probe	AAATCCCATGATCAAGCTGTCCGAACC

		[SEQ ID NO:137]
LTC4S	RT-forward	CACCACACCGACGGTACCA [SEQ ID NO:138]
	RT-reverse	TGCGCGCCGAGATCA [SEQ ID NO:139]
		CCATGAAGGACGAGGTAGCTCTA
	Taqman-forward	[SEQ ID NO:140]
	Taqman-reverse	TGCGCGCCGAGATCA [SEQ ID NO:141]
	probe	CCTGGGAGTCCTGCTGCAAGCCTACT [SEQ ID NO:142]
MRC1	RT-forward	CGCTACTAGGCAATGCCAATG [SEQ ID NO:143]
	RT-reverse	GCAATCTGCGTACCACTTGTTTT [SEQ ID NO:144]
		CGCTACTAGGCAATGCCAATG [SEQ ID NO:145]
	Taqman-forward	GCAATCTGCGTACCACTTGTTTT [SEQ ID NO:146]
	Taqman-reverse	AGCAACCTGTGCATTCCCGTTCAAGT [SEQ ID NO:147]
	probe	
MRC2	RT-forward	GGGAGCACTGCTATTCTTTCCA [SEQ ID NO:148]
	RT-reverse	CAACACATTCTCCATCTCATCCA [SEQ ID NO:149]
		GAGCACTGCTATTCTTTCCACATG [SEQ ID NO:150]
	Taqman-forward	TCTCCATCTCATCCAGGATAGACA [SEQ ID NO:151]
	Taqman-reverse	CCACCCGCTCTCTGGCAGCG [SEQ ID NO:152]
	probe	
SCYA22	RT-forward	GCATGGCTCGCCTACAGACT [SEQ ID NO:153]
	RT-reverse	CAGACGGTAACGGACGTAATCAC [SEQ ID NO:154]
	Taqman-forward	TGGCGCTTCAAGCAACTG [SEQ ID NO:155]
	Taqman-reverse	CAGACGGTAACGGACGTAATCA [SEQ ID NO:156]
	probe	AGGCCCTACGGCGCCAACAT [SEQ ID NO:157]
TNFa	RT-forward	CTGGTATGAGCCCATCTATCTGG [SEQ ID NO:158]
	RT-reverse	TTGGATGTTCTCCTCCTCAC [SEQ ID NO:159]
		GGAGAAGGGTGACCGACTCA [SEQ ID NO:160]
	Taqman-forward	TGCCAGACTCGGCAAAAG [SEQ ID NO:161]
	Taqman-reverse	CGCTGAGATCAATCGGCCCGACTA [SEQ ID NO:162]
	probe	
SCYA20	RT-forward	GGCTGTGACATCAATGCTATCATC [SEQ ID NO:163]
	RT-reverse	GTCCAGTGAGGCACAAATTAGATAAG [SEQ ID NO:164]
	Taqman-forward	TCTGGAATGGAATTGGACATAGCCCAAG [SEQ ID NO:165]

	Taqman-reverse probe	CCAACCCCAGCAAGGTTCTTTCTG [SEQ ID NO:166] ACCTCCATGATGTGCAAGTGAAACC [SEQ ID NO:167]
SCYA17	RT-forward	GGATGCCATCGTTTTGTAAGT [SEQ ID NO:168] CCTCTCAAGGCTTTGCAGGTA [SEQ ID NO:169]
	RT-reverse	GGGCAGGGCCATCTGTTC [SEQ ID NO:170] TCTCAAGGCTTTGCAGGTATTAA [SEQ ID NO:171]
	Taqman-forward	ACCCCAACAACAGAGAGTGAAGAATGCA [SEQ ID NO:172]
	Taqman-reverse probe	CCTCTCCTTGTTGGCTACCC [SEQ ID:173] CAATCTCTCAGAAAGTGAAGGG [SEQ ID:174] TCCTCTGGACCACCTCAGT[SEQ ID:175] GAACATTCCTGGGTCTGGAGTG [SEQ ID:176] TGGCCAGAAACCTCCCCGTGG [SEQ ID:177]
	IL12A	GTAAGTGAATGTCCAACGC [SEQ ID:178] GACAACTTACTGGGATGCTC [SEQ ID:179] CCAAACGCAAAGCAATACATGA [SEQ ID:180] TTTTCGCTTCCCTGTTTAGCT [SEQ ID:181] TCCAAGTGATGGCTGAATGTCGCC [SEQ ID:182]
IFN γ	RT-forward	GTTGCCTGGTCTCCTGACT [SEQ ID:183] TGTCAGCTGATCCTTCATTG [SEQ ID:184] TGAGAACAGCTGCACCCACTT [SEQ ID:185] GCTGAAGGCATCTCGGAGAT [SEQ ID:186] CAGGCAACCTGCCTAACATGCTTCG [SEQ ID:187]
	RT-reverse	ACTGCTACTGCTGCTGAGCCT [SEQ ID:188] GGTGAGGTGGATCGGTTGTAGT [SEQ ID:189] CAATCCCACGAAATCCAGGA [SEQ ID:190] TTCAGGTTGACCATCACAGTCC [SEQ ID:191] CCCAAATTCTGAGGACAAGAAGTCCCC [SEQ ID:192]
	Taqman-forward	
	Taqman-reverse	
	Probe	
IL-10	RT-forward	
	RT-reverse	
	Taqman-forward	
	Taqman-reverse	
	Probe	
IL-17A	RT-forward	
	RT-reverse	
	Taqman-forward	
	Taqman-reverse	
	Probe	

[00120] For qPCR for periostin and CEACAM5, relative copy number for periostin and CEACAM5 expression in baseline bronchial epithelial brushing samples were obtained according to a previously described method [45] and log₁₀ transformed. The 35-probe IL13 signature described in Example 9 (*see also* Fig. 11) was used as a response metric. All models were derived iteratively using the Fit Model platform in JMP 7.0. Ordinal logistic regression was performed to predict response (35 probe IL13 status) having levels (Healthy

control; HC) < (IL13 Low) < (IL13 High). The generalized predictive model for probability for each level is described as follows:

$$p_{HC} = \frac{1}{(1 + e^{(\beta_0 + \beta_1 \cdot \text{IL13})})}$$

$$p_{IL13low} = \frac{1}{(1 + e^{(\beta_0 + \beta_1 \cdot \text{IL13})})} - p_{HC}$$

$$p_{IL13high} = 1 - (p_{HC} + p_{IL13low})$$

$$\beta_0 = \sum_{i=1}^k A_i \times X_i \text{ (Linear sum)}$$

$$\beta_0 = \prod_{i=1}^k A_i \times X_i \text{ (Product for cross terms)}$$

$$\beta_0 = \text{Intercept estimate of qPCR parameter } x$$

[00121] Ordinal logistic regression was performed for the following model: (35 probe IL13 status) ~ (POSTN) + (CEACAM5). A whole model p-value of <0.0001 was derived from the dataset based on an iterative fit.

IL13 responsive genes

[00122] The relationship between periostin (also known as osteoblast specific factor) (POSTN: 210809_s_at), CLCA1 (also known as chloride channel, calcium activated, family member 1) (CLCA1: 210107_at), and SERPINB2 (also known as serpin peptidase inhibitor, clade B (ovalbumin), member 2) (SERPINB2: 204614_at) expression level was confirmed using the Wilcoxon Rank Sum test. POSTN expression level was used to categorize baseline asthma samples. A cutoff of 800 units was used, resulting in 21 asthma baseline asthma samples being classified as “IL13 low” (POSTN < 800 units) and the remaining 21 samples as “IL13 high” (POSTN > 800). Wilcoxon Rank Sum test followed by false discovery rate analysis (qvalue < 0.05) [24] identified 35 probes differentially expressed among the two groups. Hierarchical clustering using these probes was undertaken. Due to the presence of many cystatin and serpin family genes in the list differentially regulated probes, additional cystatin and serpin family probes were identified and used in an additional cluster analysis. All statistical analyses were performed using R. Microarray cluster analysis was performed using Cluster and visualized using Java Treeview [25, 26].

Serum analyte assays

[00123] Serum IgE was measured by UCSF clinical laboratories or by ELISA using a human serum IgE ELISA kit according to manufacturer's instructions (Bethyl Laboratories). Serum CEA was measured using a human serum CEA ELISA kit according to manufacturer's instructions (Alpco Diagnostics). We developed an electrochemiluminescent assay (ECLA) to measure serum periostin using anti-periostin antibodies (R&D systems).

Briefly, monoclonal anti-periostin was coated onto plates at 1.5 micrograms/ml in sodium carbonate buffer, pH 9.6 overnight at 4°C. Plates were blocked in assay buffer (1X PBS pH 7.4, 0.35 M NaCl, 0.5% BSA, 0.05% Tween 20, 0.25% CHAPS, 5mM EDTA, 15PPM Proclin) + 3% BSA for 2 hours at room temperature, then washed 4x with TBST (Tris-buffered saline + 0.1% Tween-20). Serum was diluted 1:5 in assay buffer and incubated with agitation at room temperature for 2 h, then washed 4x with TBST. Recombinant periostin (R&D Systems) was used to establish a standard range. Biotinylated polyclonal anti-human periostin (1.5 microgram/ml) (R&D Systems; biotinylated *in vitro* according to standard methods known in the art) and Ruthenium-streptavidin (0.75 microgram/ml) (Meso Scale Devices) were added in assay buffer + 5% goat serum and incubated for 90 minutes at room temperature. Reading buffer (Meso Scale Devices) was added and electrochemiluminescence was read (Meso Scale Devices). Dynamic range was 5-2000 ng/ml.

Example 2 – IL-4/13 signature and subsets of asthmatics

[00124] To determine if three IL-13 induced genes (periostin, CLCA1, and serpinB2) reflect a broader pattern of gene expression in asthmatic airway epithelium, we examined whether their expression was co-regulated at baseline within individual subjects among the 42 asthmatics studied. In pairwise comparisons, the expression levels of periostin, CLCA1, and serpinB2 were significantly correlated within individual asthmatics. Furthermore, these genes were highly expressed in some, but not all, of the asthmatic subjects (Figs. 1A and 1B). In addition, expression levels of these three genes were highly correlated within individual subjects with asthma (Fig. 1B). These data suggest that certain IL-13 markers are over-expressed in a specific subset of patients with asthma. In further experiments, we sought to identify additional genes or markers that might be directly or indirectly regulated by IL-13 and we sought to characterize subsets of asthma patients based on expression of IL-13 markers.

[00125] To identify other genes or markers that could potentially be regulated directly or indirectly by IL-13 in asthmatic airway epithelium, we examined the entire microarray dataset across the 42 asthmatic subjects for genes whose expression was significantly correlated with that of periostin. We identified a cluster of 653 probes whose expression was coregulated with periostin in individual subjects below a threshold q-value of 0.05. Unsupervised clustering of all subjects including healthy controls and asthmatics based on expression levels of those 653 probes revealed two major clusters: a cluster with high

expression levels of periostin and co-regulated genes and a cluster with low expression levels of periostin and co-regulated genes. The core of this gene cluster (Fig. 1C, right panel) comprises a subset of 35 probes representing the genes shown in Figure 13, which we refer to herein as “IL-4/13 signature,” “IL-4/13 gene signature,” “IL-13 signature,” or “IL-13 gene signature.” As indicated previously, those terms are used synonymously herein. The cluster with high expression of periostin and co-regulated genes comprised 21 asthmatic subjects and no healthy controls (Fig. 1C, right panel, labeled “IL-4/13 signature high”) whereas the cluster with low expression of periostin and co-regulated genes comprised the remaining 21 asthmatics (Fig. 1C, right panel, labeled “IL-4/13 signature low”) interspersed with all 27 of the healthy controls (Fig. 1C, right panel).

[00126] Cluster 1 (“IL-4/13 signature high”) is characterized by high expression levels of the genes corresponding to probes for periostin, CST1, CST2, CST4, CCL26, CLCA1, CDH26, PRR4, serpinB2, serpinB10, CEACAM5, iNOS, C2ORF32, PTGS1, P2RY14, RUNX2, SH3RF2, WLRW300, DNAJC12, ALOX15, GSN, RGS13, TGSAB1, PTSG1, FCER1B, and CPA3 and consists of approximately half the asthmatics in the study (N=23 out of 42 asthmatics) and one healthy control out of 27 total healthy controls. Cluster 2 (Healthy controls and “IL-4/13 signature low”) is characterized by low expression levels of the genes corresponding to the indicated probes and consists of the remaining 19 asthmatics and 26/27 healthy controls. Probes corresponding to genes predominantly expressed in mast cells, including RGS13, PTSG1, TPSAB1, FCER1B, CPA3, and SLC18A2 are indicated in blue in Table 2 and probes corresponding to genes predominantly expressed in eosinophils, including P2RY14 and ALOX15 are indicated in orange. Although the epithelial brushings consisted of predominantly epithelial cells and goblet cells (mean 97%, median 98%, minimum 91%), small numbers of infiltrating mast cells and eosinophils were observed in the brushings from cluster 1 asthmatics, and the presence of mast cell and eosinophil genes in the signature likely reflects this infiltration.

[00127] To characterize subsets of subjects with asthma based on expression of IL-13 markers, we performed unsupervised hierarchical clustering of all 70 subjects (42 asthmatics and 27 healthy controls) based on the microarray expression levels of periostin, CLCA1, and serpinB2 (Figure 1D). In this analysis, approximately half of subjects with asthma (N=22) showed consistently high expression levels of IL-13-induced genes and grouped together in one major branch of the cluster dendrogram (cluster 1, the “IL-13 high” subset). Remarkably, although periostin, CLCA1, and serpinB2 were significantly over-expressed when comparing

all 42 asthmatics to all 27 healthy controls [8], nearly half of the asthmatics examined in this study (N=20) were indistinguishable from healthy controls on the basis of expression of these three genes. This subset of asthmatics (the “IL-13 low” subset) and all the healthy controls grouped together in the second major branch of the dendrogram (Figure 1D, cluster 2). Thus, hierarchical clustering based on epithelial gene expression identified two distinct subsets of patients with asthma, referred to herein as “IL-13 high” subset and “IL-13 low” subset.

[00128] To confirm the validity of these asthma patient subsets, identified using IL-13 inducible marker expression in epithelial cells, we measured the expression level of IL-13 and certain other Th2 cytokines (i.e. IL-4 and IL-5) in bronchial biopsies obtained contemporaneously from 48 of the subjects (14 healthy controls, 18 cluster 1 asthmatics, and 16 cluster 2 asthmatics). Using qPCR, we found that IL-13, IL-5 and IL-4 expression was detectable in homogenates of bronchial biopsies. Notably, IL-13 and IL-5 expression, but not IL-4 expression, were significantly higher (Fig. 1E, *, $p < 0.002$) in cluster 1 asthmatics compared to cluster 2 asthmatics or healthy controls. There were no significant differences, however, in IL-4, IL-5, or IL-13 expression between asthmatics in cluster 2 and healthy controls (Fig. 1E). In addition, we found that expression levels of IL-13 and IL-5 were highly correlated across all of the subjects with asthma (Spearman’s rank order correlation $\rho=0.58$, $p<0.0001$; Fig. 1E). IL-4 shares a dominant signaling pathway with IL-13 and has been shown to induce periostin [7, 9] and CLCA1 [12] expression similarly to IL-13. As elevated levels of IL-4 expressing T cells have been reported in bronchoalveolar lavage (BAL) fluid [79] from asthmatics and we did not specifically examine cytokine gene expression in BAL T cells or cytokine protein levels in BAL or bronchial tissue in this study, we cannot rule out the possibility that the observed induction of periostin, CLCA1, and serpinB2 is due in part to IL-4 as well as to IL-13. Based on the data shown herein, we can confidently discern a correlation between bronchial IL-13 expression and epithelial periostin, CLCA1, and serpinB2 expression. Thus, we use the terms “IL-4/13 high” and “IL-13 high” synonymously to refer to cluster 1 asthmatics and we use the terms “IL-4/13 low” and “IL-13 low” synonymously to refer to cluster 2 asthmatics. It is understood that when the terms “IL-13 high” and “IL-13 low” are used, IL-4 and/or other as yet unidentified factors may also contribute in part to the observed gene expression patterns.

Example 3 – Constituent Genes of IL-4/13 signature

[00129] Within the IL-4/13 signature, there are two major groups of genes: epithelial or goblet cell expressed genes and mast cell expressed genes. Greater than 90% of cells in each bronchial brushing sample were bronchial epithelial cells or goblet cells (mean 97%, median 98%, minimum 91%). Expression levels of probes corresponding to the following epithelial or goblet cell genes were most significantly co-regulated with those of periostin; CST1, CST2, CCL26, CLCA1, PRR4, serpinB2, CEACAM5, and iNOS (Table 2, indicated with asterisks; > 3-fold higher expression in IL-4/13 signature high vs. IL-4/13 signature low subjects). The mouse orthologue of CLCA1, mCLCA3 (also known as gob-5) has been previously identified as a gene associated with goblet cell metaplasia of airway epithelium and mucus production; both are induced by Th2 cytokines including IL-9 and IL-13 [12-14]

Table 2.

Probe	Gene Name	Fold change, High vs. Low	p-value, High vs. Low	q-value, High vs. Low	Healthy Mean	IL-4/13 signature Low mean	IL-4/13 signature High mean	Fold change, High vs. Control	Fold change, Low vs. Control
1555778_a_at	POSTN*	11.35	2.60E-11	7.11E-07	14.93	15.73	178.51	11.96	1.05
206224_at	CS1T1*	11.12	9.09E-06	0.021609818	8.76	32.37	360.02	41.12	3.70
223710_at	CCL26*	10.22	2.88E-05	0.045024394	6.33	3.87	39.57	6.25	0.61
208994_at	CS1T1*	9.98	4.90E-06	0.014874475	10.38	67.81	676.94	65.22	6.53
210107_at	GLCA1*	9.77	1.96E-07	0.001785296	29.61	95.06	928.81	31.37	3.21
208555_x_at	CS1T2*	9.13	9.04E-07	0.004119975	5.13	14.75	134.71	26.26	2.88
210809_s_at	POSTN*	7.70	3.72E-12	2.03E-07	260.24	334.28	2572.46	9.88	1.28
204919_at	PRR4	6.09	5.73E-06	0.01649484	37.33	97.20	592.05	15.86	2.60
207741_x_at	TPSD1	4.76	1.54E-06	0.005250514	9.96	18.81	89.64	9.09	1.91
204614_at	SERPINB2*	4.52	4.30E-07	0.002615287	97.43	212.63	960.75	9.86	2.18
201884_at	CEACAM5*	3.48	4.30E-07	0.002615287	426.04	525.17	1830.00	4.30	1.23
210037_s_at		3.30	2.51E-05	0.045024394	6.39	6.54	21.60	3.38	1.02
216485_s_at	TPSG1	3.23	7.81E-06	0.0203328	10.04	17.84	57.65	5.74	1.78
216474_x_at	TPSD1	3.06	1.60E-07	0.00174608	46.63	77.82	238.00	5.10	1.67
205883_x_at	TPSD1	2.97	2.72E-06	0.0049597	53.99	76.74	227.95	4.22	1.42
225316_at	MFS2	2.96	2.18E-05	0.042590277	29.03	26.00	76.95	2.65	0.90
205624_at	CPA3	2.94	3.55E-07	0.002615287	99.69	166.29	489.17	4.91	1.67
206637_at	GPR105	2.88	6.27E-07	0.003117623	40.90	65.93	189.66	4.64	1.61
223206_at	CDH26	2.85	1.29E-06	0.00470447	223.92	326.79	932.02	4.16	1.46
207134_x_at	TPSD1	2.66	6.27E-07	0.003117623	50.28	86.08	228.78	4.55	1.71
210084_x_at	TPSD1	2.56	6.70E-06	0.018307462	48.91	77.59	198.54	4.06	1.59
200896_s_at	GSN	2.50	2.88E-05	0.045024394	246.87	224.72	562.74	2.28	0.91
226751_at	C2ORF32	2.50	9.09E-06	0.021609818	35.39	32.96	82.47	2.33	0.93
238429_at	TRACH2000196	2.39	2.88E-05	0.045024394	36.79	37.68	89.91	2.44	1.02
218976_at	DNAJC12	2.32	2.18E-05	0.042590277	48.54	38.92	90.11	1.86	0.80
217023_x_at	TPSD1	2.31	1.29E-06	0.00470447	53.13	67.76	156.32	2.94	1.28
215382_x_at	TPSD1	2.30	1.08E-06	0.00459197	38.96	52.95	121.86	3.13	1.36
210259_at	RGS13	2.25	2.88E-05	0.045024394	8.75	7.56	17.04	1.95	0.86
205857_at	SLC18A2	2.23	1.05E-07	0.001435039	84.08	100.96	225.07	2.68	1.20
245339_at	SERPINB10	2.15	1.06E-05	0.024063758	42.37	40.04	86.16	2.03	0.95
243582_at	SHRF2	2.00	1.89E-05	0.039905857	82.64	85.89	171.90	2.08	1.04
207496_at	FCER1B	1.92	2.51E-05	0.045024394	37.55	37.03	71.28	1.90	0.99
232321_at	RUNX2	1.77	2.88E-05	0.045024394	288.42	299.21	529.59	1.84	1.04
236669_at	PTGS1	1.73	4.18E-06	0.013429003	82.69	88.43	152.98	1.85	1.07
207328_at	ALOX15	1.72	1.64E-05	0.03598964	812.57	895.94	1538.53	1.89	1.10

[00130] SerpinB2 is a member of a large family of serine protease inhibitors encoded in a gene cluster on chromosome 18q21 (Fig. 2A, top; screen capture from UCSC Genome Browser at <http://genome.ucsc.edu>). Expression levels of serpins B2 [8], B3, and B4 are induced in airway epithelial cells upon stimulation by recombinant IL-4 and IL-13 [7, 15].

[00131] Cystatins (CST) 1 and 2 are members of a large family of cysteine protease inhibitors encoded in a gene cluster on chromosome 20p11 (Fig. 2A, middle; screen capture from UCSC Genome Browser at <http://genome.ucsc.edu>). Several cystatins are expressed in bronchial epithelium [16]; CST4 has been identified at elevated levels in bronchoalveolar lavage fluid (BAL) of asthmatics [17]; serum CST3 is elevated in asthmatics relative to healthy controls and its levels are decreased by ICS treatment [18]. As serpin and CST gene families are each colocalized on the chromosome, we explored whether any additional members of the serpin and cystatin gene families are co-regulated with those already identified. We performed unsupervised clustering of the microarray data, restricted to serpin and cystatin gene families. We found that serpins B2, B4, and B10; and cystatins 1, 2, and 4 were significantly co-regulated, with the highest expression levels occurring in asthmatics positive for the "IL-4/13 signature" (Fig. 2B).

[00132] PRR4 is a member of a large family of proteins encoded in a gene cluster on chromosome 12p13 (Fig. 2A, bottom; screen capture from UCSC Genome Browser at <http://genome.ucsc.edu>). These proline-rich proteins are found in mucosal secretions including saliva and tears. Related, but non-orthologous proteins SPRR1a, 2a, and 2b have been identified in bronchial epithelium in a mouse model of asthma and are induced by IL-13 [19, 20]. Proline-rich proteins from the PRR/PRB family have been identified in bronchial secretions [21] and their expression has been documented in bronchial epithelium [16]. Of the PRR/PRB family, PRR4 and PRB4 were significantly upregulated in asthmatics with high expression of the IL-4/13 gene signature (Fig. 2C, left and middle).

[00133] CCL26 (Eotaxin-3) is an IL-4 and IL-13 inducible chemokine in asthmatic airway epithelium.

[00134] CEACAM5 encodes a cell-surface glycoprotein found in many epithelial tissues and elevated serum. CEACAM5 (carcinoembryonic antigen; CEA) is a well-documented systemic biomarker of epithelial malignancies and metastatic disease. Elevated CEA levels have been reported in a subset of asthmatics, with particularly high serum levels observed in asthmatics with mucoid impaction [22]. CEACAM5 is significantly upregulated in IL-4/13 signature high asthmatic airway epithelium compared to IL-4/13 signature low and healthy

control airway epithelium (Fig. 2C, right), which suggests that serum CEA levels may be used to distinguish between these two asthmatic sub-phenotypes.

[00135] Inducible nitric oxide synthase (iNOS) is associated with airway inflammation and is induced by IL-13 in human primary bronchial epithelial cell cultures [23]. The measurement of exhaled nitric oxide (eNO), a product of iNOS enzymatic activity, is commonly used in the diagnosis and monitoring of asthma.

Example 4 - Mast Cells

[00136] Although the airway brushings used in this study comprised predominantly epithelial and goblet cells, there were small but significant percentages of infiltrating leukocytes in many of the samples. Genes whose expression is specific to mast cells, including tryptases (TPSD1, TPSG1), carboxypeptidase A3 (CPA3), and FcεpsilonRIβ, were significantly correlated with the IL-4/13 gene signature (Table 2 and Table 4, mast cell genes marked with double astericks in Table 4). Given the significant role of tissue-resident mast cells in allergic disease and the recent observation that the presence of IL-13 expressing mast cells in asthmatic endobronchial biopsy specimens is positively correlated with detectable levels of IL-13 in sputum [6], the high correlation between mast cell-specific genes and the IL-4/13 signature suggests that: 1) mast cells may be a significant source of IL-13 in the airway epithelium and 2) mast cell infiltration into airway epithelium may be a unique feature of the IL-4/13 signature high subset of asthmatics.

Example 5 – Combinations that Predict IL-4/13 Signature

[00137] Expression levels of individual genes in the IL-4/13 signature may predict the IL-4/13 signature status of individual subjects with variable accuracy; however combinations of these genes may be used to assign individual subjects to the IL-4/13 signature high or low category with increased sensitivity and specificity.

Example 6 – Steroid Effect

[00138] The standard of care for bronchial asthma that is not well-controlled on symptomatic therapy (i.e. beta-adrenergic agonists) is inhaled corticosteroids (ICS). In mild-to-moderate asthmatics with elevated levels of IL-13 in the airway [6] and in eosinophilic

esophagitis patients with elevated expression levels of IL-13 in esophageal tissue [11], ICS treatment substantially reduces the level of IL-13 and IL-13-induced genes in the affected tissues. In airway epithelium of asthmatics after one week of ICS treatment and in cultured bronchial epithelial cells, we have shown that corticosteroid treatment substantially reduces IL-13-induced expression levels of periostin, serpinB2, and CLCA1 [8]. Further examination of the genes listed in Table 2 revealed that, in the 19 subjects in our study who received one week of ICS treatment prior to a second bronchoscopy, the vast majority of IL-4/13 signature genes was significantly downregulated by ICS treatment in asthmatic bronchial airway epithelium (periostin shown as an example, Fig. 3A). This downregulation could be the result of ICS-mediated reduction of IL-13 levels, ICS-mediated reduction of target gene expression, or a combination of the two. However, two genes in the IL-4/13 signature, PRR4 (Fig. 3B) and RUNX2 (Fig. 3C), were not substantially downregulated in individual subjects after one week of ICS treatment. This suggests that PRR4 and RUNX2 may be steroid-insensitive markers of the IL-4/13 signature in asthmatic airway epithelium. Another possibility is that PRR4 and RUNX2 are only indirectly regulated by IL-4 and/or IL-13; for example, as PRR4 is found in many secretions, it may be a goblet cell-specific gene. As goblet cell differentiation from epithelial cells is induced by IL-13, ICS-mediated inhibition of IL-13 and IL-13 dependent processes may not substantially impact on goblet cell number after only 7 days of treatment, but after longer-term ICS treatment, goblet cell numbers (and hence PRR4 expression in endobronchial brushings) may be expected to decrease. In severe asthmatics who are refractory to ICS treatment, a similar fraction of subjects (approximately 40%) was found to have detectable sputum IL-13 levels to that seen in mild, ICS-naïve asthmatics [6], which is consistent with the fraction of subjects with the IL-4/13 signature observed in this study. This observation suggests that, although the IL-4/13 signature is significantly downregulated by ICS treatment in the mild-moderate, ICS-responsive asthmatics examined in the present study, it may still be present in severe steroid-resistant asthmatics.

Example 7 – Relationship of IL-4/13 signature to clinical features and other biomarkers

- Demographics

[00139] Eosinophilic asthma, as defined by elevated levels of airway eosinophils, is associated with atopy and occurs with approximately equal prevalence between males and females, while the non-eosinophilic phenotype, as defined by a relative absence of

eosinophils in the airway and associated with a lack of atopy, shows a significant female predominance [1]. Of the subjects classified according to the airway epithelial IL-4/13 gene signature, 10/21 (48%) IL-4/13 signature high subjects were female while 15/21 (71%) IL-4/13 signature low subjects were female (Table 3). There was no significant skewing by self-reported ethnicity between the IL-4/13 signature low and high groups.

Gender distribution of IL-4/13 signature, N (%)

Category	F	M
LOW	15/(71)	6(29)
HIGH	10(48)	11(52)
CONTROL	15(54)	13(46)

- FEV₁ and Methacholine responsiveness

[00140] While the gender skewing between the IL-4/13 low and high groups suggest that the observed gene expression patterns in asthmatic airway epithelium reflect stable underlying phenotypes, it is possible that the observed gene expression patterns merely reflect disease severity or activity at the time of bronchoscopy. To determine whether the IL-4/13 signature was correlated to asthma severity, we compared forced expiratory volume in one second (FEV₁, as a percentage of predicted from patient weight, measured at a screening visit one week prior to bronchoscopy) between the groups and found that, while both the IL-4/13 signature high and low groups had significantly lower FEV₁ than healthy controls, there was no statistically significant difference between the groups (*see* Fig. 5A), although there were more subjects that might be classified as “moderate” (i.e. FEV₁ 60-80% predicted) in the IL-4/13 signature high group than in the low group. The minimal concentration of methacholine in mg/ml required to induce a decrease in FEV₁ of 20% (PC₂₀, measured at a screening visit one week prior to bronchoscopy) is a measure of bronchial hyperresponsiveness. This is a measure of bronchial hyper-reactivity (BHR). Both the IL-4/13 signature high and low groups had significantly lower PC₂₀ values than healthy controls; while there was a trend toward lower PC₂₀ values in the IL-4/13 signature high group than in the low group, this difference did not reach statistical significance (*see* Fig. 5C).

- IgE and Eosinophils (peripheral and airway)

[00141] To determine whether the IL-4/13 signature status of an individual subject could be predicted by standard measures of atopy, we examined levels of serum IgE (international

units per milliliter; 1 IU = 2.4 ng), peripheral blood eosinophil counts (absolute number of eosinophils $\times 10^9$ per liter of blood), and eosinophil percentages in bronchoalveolar lavage fluid (BAL) (percentage of eosinophils relative to the total number of non-squamous cells in bronchoalveolar lavage fluid) using standard clinical laboratory tests, obtained at the time of bronchoscopy. When subjects were stratified for IL-4/13 signature status, there were significant differences in serum IgE (*see* Fig. 6B), peripheral blood eosinophil counts (*see* Fig. 6C), and BAL eosinophil percentage (*see* Fig. 6D), with significantly higher values for each analyte observed in the IL-4/13 signature high group relative to the low group. Taken individually, neither IgE level nor peripheral blood eosinophil count predicts the airway epithelial IL-4/13 signature status of any individual subject with simultaneously high sensitivity and specificity. However, among individual asthmatics, IgE level and peripheral blood eosinophil counts are weakly but significantly correlated ($\rho=0.44$, $p=3.4 \times 10^{-3}$). When considered as a composite, empirically derived cutoff values of both 100 IU/ml IgE and 0.14×10^9 /L eosinophils predict the airway epithelial IL-4/13 signature status of individual subjects with high sensitivity and specificity (Fig. 4; 18/21 correct for both low and high IL-4/13 signature; sensitivity = 86%, specificity = 86%).

	<p>GTACACACACCCC [SEQ ID NO:33]</p> <p>206224_at: GGAGGATAGGATAATCCGGGTGGCATCTATAACGACAGCTCAATGATGAGTGGGTACA GCGTCCCTTCATCTCCATCAGCGATATAACAAGCCACCAAGATGACTACTACAG ACGTCCGCTGCGGTACTAAGAGCCAGGCAACAGCCGTTGGGGGTGAATTACTTCTT CGACGTAGAGGTGGGCGGAACCATATGTACCAAGTCCCAAGCCCAACTTGGACACCTGTGC CTTCCATGAACAGCCAGAACTGCAAGAGAAACAGTGTGCTCTTTTCGAGATCTACGAAGT TCCTGGGAGAACAGAAAGGTCCCTGGTGAATCCAGGTGTCAAGAACTCTAGGGATCTGT GCCAG [SEQ ID NO:34]</p>
CCL26	<p>223710_at: GAGAAAGGCCGTGATTGTGACATCATGATGGGCCCTCTCCTTGGCCTCTGCTGTGCTCTG GCCTCCCTCTGAGTCTCCACCTTGAACCTGCCACAGTGGGAGTGACATATCCAAGACC TGCTGCTTCCATACAGGCCAAGCCCTTCCCTGGACTGGGTGGGAAGCTATGAATTC ACCAAGTAACAAGTGTCTCCAGCGGCTGTATATTCACCTACCAAAAAGAGGCAAGAAAGTC TGTACCCATCCAAAGGAAATAAATGGGTGCAAAAATACATTTCTTACTGAAAACTCCGAAA CAATGTGACTACGTGAATTTTCATCCGAGGACGCTTGGACCCCGCTCTTTGGCTCTGCA GCCCTCTGGGGAGCCTGCGGAAATCTTTTCTGAAGGCTACATGGACCCGCT [SEQ ID NO:35]</p>
CLCA1	<p>210107_at: GGCCAAATCACCGACCTGAAGGGGAAAATTCACGGGGGCGAGTCTCATTAATCTGACTTGG ACAGCTCCTGGGGATGATTATGACCATGGAAACAGCTCACAGTATATCATTCGAATAAGT ACAGATATCTTGTATCTCAGAGACAAAGTCAATGAATCTCTTCAAGTGAATACTACTGCT CTCATCCAAAGGAAGCCAACTCTGAGGAAGTCTTTTGTTTAAACCCAGAAAACATTA TTTGAAAATGGCACAGATCTTTTCATTTGCTATTCAGGCTGTGATAAGGTCGATCTGAAA TCAGAAATATCCAACTTGCACGAGATCTTTTGTATTTATTCCTCCACAGACTCCGCCAGAG ACACTAGTCTCGATGAACGCTGCTCTCTCTCTAAATATTCATATCAACAGCAACAT CTGGGCATTCACATTTTAAAAATATGTGGAAAGTGGATAGGAGAACTCGACGCTGTCAATA GCCTAGGGC [SEQ ID NO:36]</p>

CST2	<p>208555 x at: GAGCCCAGGAGGAGGACAGGATAATCGAGGGTGGCATCTATGATGCAGACCTCAATGA TGAGCGGTACAGGTGCCCTTCATCTTTGTATCAGCGAGTATAACAMGGCCACTGAAGA TCAGTACTACAGACGCCCTGCTCGGGTGCTACGAGCCAGGAGCAGATCTGGCGGGGT GAATTACTTCTTCGACATAGAGTGGCGGAACCATATGTACCAAGTCCCGCCCAACTT GGACACCTGTGCTTCATGAACAGCCAGAACTGCAGAAAGAACAGATGTGTGCTCTTTCCA GATCTACGAAGTTCCCTGGGAGGA [SEQ ID NO:37]</p>
PRR4	<p>204919 at: AAGACTTTACTTTCACCATACCCAGATGTAGAGGACTCAAGTCAGAGACCAGATCAGGGAC CCCAGAGACTCTCTCTGAGAGGACTCTACCTAGACCCCTGGTGATAGTGTAAACCAG ATGATGTCCTCAGCAGAGACCAACAAACAGGAGGCCATCACCGCCATCTCTCCCCAC CTCCTTTCAAATCAGCAACGACCAACCCCAACGAGACACCGTCAACTCTCTTACCCC GATTCCTCTCTGTCAGCCTGCAGGAAGCATCATCTTCTCCGGAGGACAGACCAGCA GACATCCCCA [SEQ ID NO:38] Serpin peptidase inhibitor, clade B (ovalbumin), member 2</p>
SERPINB2	<p>204614 at: TTCCCTCACCTAAAACTAAGCGTGCTCTCTGCAAAAGATTTTGTAGATGAGCTGTGT GCCTCAGAAATGCTRAATTCCAAATGCCAAAATTTAGAGATGTTTTCTACATATTTCTGC TCTCTGACAACTTCTGTCTACCCCACTAAATAAAACACAGAAATPAATTAGACAAATGTC TATTATACATGACAACTTAAATCATTTGGTCTCTTAAATGGGATCATGCCCATTT AGATTTCCCTACTACAGTTATTTTATACATTAACCTTTTACTTTGTTATTATTAT TTTATATAATGGTGAGTTTAAATTTATGCTCACTGCCTATTTAATGTAGCTAATAAAG TTATAGAGCAGATGATCTGTTAATTTCTCTATTAATAAGTCCCTTAAATTTGTTCTCATA ATGAAGAAATAGTAGGTACCCCTCCATGCCCTTCTCTGTAATAATAT [SEQ ID NO:39]</p>
CEACAM5	<p>201884 at: AGAAGACTCTGACTGTACTCTTGAATACAAAGTTTCTGATACCACCTGCATGCTGAGAA TTTCCAAAACCTTAATGAACATACTGACAGCTTCATGAACCTGTCACCAAGATCAAGCA GAGAAAATAATTAATTTTCATGGGACTAAATGAACATAATGAGGATTGCTGATCTTTAAAT</p>

iNOS	<p>GTCTTGTTCCAGATTTCAGGAAACITTTTCTTTTAAGCTATCCACTCTTACAGAA TTTGATAAAATATCTTTGTGAACAAAAATTGAGACATTTACATTTTCTCCCTATGTGG TCGCTCCAGACTTGGGAAACTAT [SEQ ID NO:40]</p> <p>Inducible nitric oxide synthase</p> <p>210037_s_at: TCATCGGGCTGGCACAGGCAATCGCGCCCTTCGCAGTTTCTGGCAGCAACGGCTCCATG ACTCCAGCACAGGAGTGGGGAGCGGCATGACCTTGGTGTTTGGGTGCCCGCCGCC CAGATGAGACCACTATCTACCAGGAGATGCTGGAGATGGCCAGAAAGGGGTGCTGC ATCGGTGCACACAGCTATTCCCGCTCGCTGCAGGCCAAGCTCTATGTTACAGGACA TCCTGGCGCAGCAGTGGCAGCGAGGTCTCCGTGCTGCCACAAGGAGCCAGGCCACC TCTATGTTTGGGGGATGTGGCAATGGCCGGGACGTGGCCACACCTTGAAGCAGCTGG TGGCTGCCAAGCTGAAATTGAAATGAGAGCAGGTGCGAGGACTATTCTTCAGCTCAAGA GCCAAGCGCTATCACGAAGATATCTTTGGTGTGTATTTCCCTTACGAGGGCAAGAGG ACAGGTGGCGGTGCAGCCC [SEQ ID NO:41]</p>
SERPINB4	<p>210413_x_at: GTGATTTTACACTTACCTTCGGTTTCAAAATGGAAGAGAGCTATGACCTCAAGGACACGTTG AGAACATGGGAATGGTGAATATCTTCAATGGGGATGCGAGACCTCTCAGGCATGACCTGG AGCCACGGTCTCTCAGTATCTAAAGTCTCTACAAAGGCCCTTGTGGAGGTCACTGAGGAG GGAGTGGAGCTGCGAGCTGCCACCGCTGTAGTAGTGGTGAATATCATCTCTCTCACT AATGAAGAGTTCTGTGTAATCACCCCTTCTCTATCTTCATAAGGCAAAATAAGACCAAC AGCATCTCTCTTATGGCAGATTCTCATCCCATAGATGCAATAGTCTGTCACTCCCAIT TAG [SEQ ID NO:42]</p> <p>211906_s_at: GATACGACACTGGTCTTGTGTAACGCAATCTATTTCAAGGGCAGTGGGAAGAATAATTT AAAAAGAAAACAATAAGAGGAAAAATTTGGCCAAACAGGATGTACAGGCCAAGGTC CTGGAATACCATACAAAGGCAAGATCTAAGCATGATTGTCTGTGCCAATGAATC GATGGTCTCGAAGAGCTGAAGAGAACTCACTGCTGAGAAATGATGGAATGGACAAGT</p>

	<p>TTGAGAATATGAGAGAGACATGTGTGCAATTTACACTTACCTCGGTTCAAAATGGAAGAG AGTATGACCTAAGGACACGTTGAGAACCATGGGAATGGTGAATATCTCAATGGGGAT GCACACCTCTCAGGCATGACCTGGAGCCACGGTCTCTCAGTATCTAAAGTCCCTACACAAG GCCTTGTGGAGGTCACTGAGGAGGAGTGGAAAGCTGCAGCTGCCACCGCTGTAGTAGTA GTCGAATTATCATCTCTTCAACTAATG [SEQ ID NO:43]</p>
CST4	<p>Cystatin-4</p> <p>206994 at: GCGAGTACAACAAGGCCACCGAAGATGAGTACTACAGACGCCCGCTGCAGGTGCTGCGAG CCAAGGAGCAGACCTTTGGGGGGTGAATTAATCTTTCGACGTAGAGTGGGCCGCACA TAATGTACCAAGTCCACGCCAACTTGGACACCTGTGCTTCATGAACAGCCAGAAGCTGC AGAAACAACAGTTATGCTCTTTCGAGATCTACGAAGTTCCCTGGGAGGACAGAAATGTCCC TGGTGAATTCACAGTGTCAAGAAGCCTAGGGGTCTGTGCCAGGCCAGTCAACCGGACAC CACCCACTCCACCCCTGTAGTGTCTCCACCCCTGGACTGTGGCCGCCACCCCTGCGGG AGGCTCCCCATGTGCTGTGCAAGAGACAGACAGAGAGAGGCTGCAGGAGTCTCTTGT GCTCAGCAGGGCGCTCTGCCCTCCCTCTCTCTTGCTTCTAATAGACCTGGTACATG GTACACACACCCC [SEQ ID NO:44]</p>
PRB4	<p>proline-rich protein BstNI subfamily 4 precursor</p> <p>216881 x at: CCACCTCTCCAGGAAAGCCAGAAAGACCCACCCACAGAGGAGTAACCAGTCCCAAGGT CCCCACCTCATCCAGGAAAGCCAGAAAGGACACCCCAAGGAAAGAAACAAAGTCCCGA AGTCCCGATCTCTCCAGGAAAGCCAGGACACCCCAAGGAAAGGAAAGGAAAGGAAAGGCT CAAGGTCCCACTCTCTGAAAGCCAAAGGCCACCCCAAGGAGGCAATCCCAAG CAGCTCAGGCACTCTGCTGGAAGGCCCAAGGGGGACCTCCACCTCTCAAGGGGGC AGGCCACCAAGCTGCCAGGACAAAGCTCCCAAGTAACTAGGATTCATATGACAG GAAGTGAATAAGAAATATCAGTGAATTCAAATATTCATTTGCTACAAATGCCGTGACA TTGGAACAAGGCAATCATAGCTCTAAC [SEQ ID NO:45]</p>
TPSD1**	<p>207741 x at:</p>

	<p> TGACGCAAAATACCACTTGGCGCTACACGGGAGACGATCCGCATCATCCGTGACGA CATGCTGTGTCCGGGAACAGCCAGAGGACTCTGCAAGGGCGACTCTGGAGGGCCCT GGTGTGCAAGTGAATGGCACTGTGCTACAGCGGGGTGTGTCAGCTGGGACGAGGCTG TGCCAGCCCAACCGCGCTGGCATCTACACCGGTGTCACCTACTTGGATGGATGATCCA CCACATATGTCCTCCAAAGCCGTGAGTCAGGCCCTGGGTGTGCCACCTGGGTCACTGGAGG ACCA [SEQ ID NO:46] </p> <p> Affy 216474 x at CCGCATTTTCTCTGAAGCAGGTGAAGGTCCCATATGGAAACCACATTTGTGACGCA AAATACCACCTTGGGCGCTACACGGGAGACGACGTCCGCATGTCCTGTGACGACATGCTG TGTCCGGGAACATCCGAGGACTCATGCAAGGCGACTCCGAGGGCCCTTGTGTGC AAGGTGAATGGACCTGGCTGCAAGCGGGCTGGTCACTTGGGGCGAGGGCTGTGCCAG CCCAACCGGCTGGCATCTACACCGGTGTCACCTACTTGGACTGGATCCACCATAT GTCCCAAAAAGCGGTGAGTCAGGCTGGGTGTGCCACCTGGGTCACTGGAGGACCAA [SEQ ID NO:47] </p> <p> 205683 x at: TGACGCAAAATACCACTTGGCGCTACACGGGAGACGACGTCCGCATCGTCCGTGACGA CATGCTGTGTCCGGGAACACCGAGGACTCATGCCAGGGCGACTCCGAGGGCCCT GGTGTGCAAGTGAATGGCACTGGCTGCAAGCGGGCTGTGTCAGCTGGGCGAGGGCTG TGCCAGCCCAACCGCGCTGGCATCTACACCGGTGTCACCTACTTGGACTGGATGCA CCATATGTGTCCTCCAAAGCCGTGAGTCAGGCCCTGGGTGTGCCACCTGGGTCACTGGAGG ACCAACCTGTGTCTCCAAAACACCACTGCTTCTACCCAGGTGGCGACTGCCCCCA CCTTCCCTGCGCGCTGTGAGTGCCCTTCTGTGCTAAAGCCCTGCTCTCTCTGAGC CCGTTCCTGCTGTGAGGACCTTCTCTATCTGAGGCCCTTCCCTGTCTCTATAGCCTG ACGCTGCAACCGGCGCTCCAGCCCTCCCTGCCAGATAGCTGGTGGTGGGCGCTAATC CT [SEQ ID NO:48] </p> <p> 207134 x at: TGACGCAAAATACCACTTGGCGCTACACGGGAGACGACGTCCGCATCGTCCGTGACGA </p>
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CCCCTGCTGCAGACCTTCCCCCGGGATGCCGTGCTGGGTCACTGGTGGGGCGAT
GTGGCAATGATGAGCGCTCCCAACCGCCATTCTCTGAAAGCAAGTGAAGTGTCCCAATA
ATGGAACAACCAATTGTGACGCAAAATACCACTTGGCGCTACACGGGAGACGACGTC
CGCATCTCCGTGACGACATGTGTGTGCCGGGAAACACCCGGAGGACTCATGCCAGGTG
GCGACT
[SEQ ID NO:51]

	<p>215382_x_at: CCGGTACGACAGGATCATCGTGCACCCACACAGTTCTACATCATCCAGACTGGAGCGGATATC GCCCTGTGGAGCTGGAGAGCCCGTGACATCTCCAGCCGCTCCACACGGTCATGCTG CCCCTGCCGAGACCTTCCCCCGGNNTGCGTGTCTGGGTCACTGGCTGGGGCGAT GTGAAATAGTAGCCCTCCACCGCCATTTCCTCCCTGAAGCAGGTGAAGGTCCCTCCATA ATGGAACACCATTTGTGACGCAAAATACCACTTTGGCGCTACACGGGAGACGACGTCT CGCATATCGGTGACGACATGCTGTGTGCGGGGAACACCGGAGNNNTCATGCCAGGC GACTNAGAGGGCCCTGGTGTGCAAGGTGAATGGCACTGGCTNACGGCGGGGTGGTC AGCTGGNCGAGGGCTGTGCCAGCCACCCGCGCTGGCATCTACACCCGTGTCACTAC TACTGGACTGGATCC [SEQ ID NO:32]</p>
TPSG1**	<p>216485_s_at: GTCGTACGGACGATGCGGAGCTCGTCTCTCCGTGTAGGCCACCAAGGTGGTATTTTGGCTC ACAAATGTGGTTTTCATTATGGGACCTTCACTCTGCTTACAGAGGAAATGGCGTGGAG GCGCTCATATTGTCCACATCGCCCAAGCAGTGACCCAGCACGGCATCCCCGGGGGAA GGTCTTGAGGACGGGGCAGGTGACCGGTGGACGTGGCTGGAGACGTTTACCGGCTC CTCAGCTCCAGCAGGGCGATGTCCGCTCCGATCTGGCGGTGTAGAACTGTGGGTGCAC GATGATCTGCTGACCGGCAGCAGCTGGTCTCTGGTAGTACAGGTGCTGTCTCCCGCAGTTG CACCGTCCACGCAGTGCGCTGCGGTACACCCACTGGGGGTGGAT [SEQ ID NO:33]</p>
	<p>220339_s_at: GGTGAAAGTCTCGTGTGGACACAGAGACCTGCGCCGGGACTATCCCGGCCCGGGGG CAGCATCTTCAGCCGACATGCTGTGTGCCCCGGGGCCCCGGGGATGCCGTGCCAGGACGA CTCGGGGGCTCTGGTCTGCCAGGTGAACGGTGCTCTGGGTGCAAGGTGCAATTGTGAG CTGGGTGAGGGCTGCGGCCGCCCAACAGCCGGGAGTCTACACTCGTGTCCCTGCCCTA CGTAACTGGATCCGCGGCACATACAGCATCAGGGGGCTCAGAGTCTGGGTACCCCAAG GCTCCCTCTGCTGCTTATCTCCCGCCGCTTCTCTCTGCTAGTCTCTGTT CCTGTGGCCAGTGCCTGTGCACCCATCTCGCGGATGGTACTCCTTCCCGGCCCTGA CTGATGGCAGGAATCCAAAGTGCAATTTCTAATAAGTTACTATTATTCCTCGCTCCGCCCC CTCCTCTCTCTTGAGAAGTGAGTCTTCTGCAATCAGATT [SEQ ID NO:34]</p>

	<p>213536 s. at: TGCCACAAAGTGCCTGCTTATGAGGGCGCAAACTCTTGGCTTGCTCGGACCCCTTTTC TGCCTACCTCCTGTTTGGCAGTAATCTGTAGGCCCTCGCTGAGCTGGTCTTGG ATAATTGATATTAAGAAAGTTCCTGATTTAAATAGGAATCTGTTTGAATGTGATGGCT GGCTCCAGTCTCTGCTCCCTCTAAGATGGACGACACATGTCCTCCGAAAGGGTACACA TTCGGGTGAAATAATGGTGGTTCGAAATTACATTTGGTGGCGAAGATGGATATCATCT TTGAAAGCATCCGATTTAAACAAGCCCTCTTCCACCGAGTCCCTTTCTTCTCTGGA ATGGCGCACTCCAGTCA'GAGGTTCACTGTGCATCGGGATTTTGTGTTGGACAGCC ACGAACAAATGGGTGGTCTTCTCTCAATGCTTTCTCTCTGCGGCGAGTCTGCTGAGG GGATCCCGACATGTTCAAGTCCCTC [SEQ ID NO:53]</p> <p>214067 at: AGAGACTTTCAGGGCATACTGGGGGCTTGGCTTCTCTCACTCGCTCGATGGCCCTCAGT GTGCTCTCAAGGCTGGTGCACAAACCTTGTGGAGATAGCTGAGCAGGGCTCTCTGTC GTCCACCTGTGAGGGCCATGGTACCCGCGGTAAAGCACCTGTGTACAGGGCTCTCTC GTAGAGCATCTCCACCTCTGCGGCCAGGCTCTAGGCCGAGGCTGGGATCCACAGG CTCGGGGGTGTGGCGACCTGCGAGGGGACCTGAGGACGGCAAGCCCTGTCT GCCCTCCCTCTTTCAGCATGAGGCGCATGTGGCAAGAACTCCACGCCATCCCGGG TTTCCAGGCCCGGTGCGAGGCTCTGCGGGTGGCGCTGGCACTCCCTGGGTCTGTGCTC AGTCTGCGGGCGAAGGACGGGCAACCTGCACTGCGCTGAGCACGCTGCTCTTAATGTC CAGCAAGGTTCGACATGGCGGGTGACCCGTGG [SEQ ID NO:56]</p> <p>Major facilitator superfamily domain-containing protein 2</p>
MFSD2	<p>225316 at: TGCTGCTCTTCAAAATGTATACCCCTATGATGAGGAGAGGGCGGCGAGAAATAGAAGGCC TGCAGGCATGAGGACAGGCCACAGCTTGGCTGCTCAGAAACAGACTCCACAGAGC TGCTAGCATCTCTAGGGGGCCGACGTGGCCGAAAGCCACATGAGAAAGGCCACAGA AGGATCAGAGACCTGTCTGCCGCTGTGAGCAGCTGAGACTGACAGTGTCTAGGAAGGA ACTGAAGATCAAGAGGTGGCCAGGACATCTGCTGCTCACTGTGGGGCGGCTGCT CTGTGGCTTCTGCTCTCCCTCTGCTGCTGCTGGGGCAAGCCCTGGGGCTGCGACTGT</p>

	<p>GAA'TATGCCAAGGACTGATGGGCCTAGCCCGGAACACTAATGTAGA [SEQ ID NO:57]</p>
CPA3**	<p>Carboxypeptidase A3 205624 at TATGAACCCGCTACATCTATGGCCCAATAGAAATCAACAAATTTACCGGATATCAGGTTCT TCTTTAGACTGGGCTTATGACTGGGCATCAAAACACACATTTGGCTTTGAGCTCCGAGAT AAAGGCAAAATTTGGTTTCTCTCCAGAAATCCGGATAAAGCCAACTGCGAGAGAGACC ATGCTAGCTGCAAAATTTATGGCAAGTATATCCTCAAGCATATCTTCTAAAGAACTGCC CTCTGTTGGAATAAGCAATTAATCCTTTTGTGGCTTTCATCAGAAAGTCATCTTC AGTTATCCCAAAATGACGCTTCTATTTCACCTGAATCCTTCTCTGCTCATTTAAGTCCC ATGTTACTGCTGTTTGTCTTTTACTTACTTTAGTAGCACCATAACGAAGTAGCTTTAAGT GAAACCTTTTAACTACCTTTCTTTTGTCCAAAGTGAAGTTTGGACCCAGCAGAAAGCAITTA TTTTGAAAGGTGATATACAGTGGGGCACAGAAACAAATGAAACCCCTCAGTTTCTCACA GATTTTCACCAATGGCTTCATCAA [SEQ ID NO:58]</p>
GPR105***	<p>G-protein coupled receptor 105 206637 at: TGAGCTGGGGTCTGGTGTTAGAAATATTTTAAAGTAGGCTTTACTGAGAGAACTAAAT ATTGGCAATACGTTATCAGCAACTCCCTGTTCAATAGTATGGGAAAAATAAGATGACTG GGAAAAGACACACCCACACCGTAGAACATATATTACTACTGGCGAATGGGAAGGAG ACCATTTCTTAGAAAGCAATAACTGTATTTTAAATCTAAAAATTACATTAAATGA GTGCAATAAACAATGAAATTCACACATCACAATTTTCTGGAAAAACAGACGGA TTTTACTCTGGAGACATGGCATACGGTTACTGACTATGAGCTACCAAACTAAATCT TTCCTGCTATTAACCTGGCTAGAGACATCACTATTTTTCAAAATGTTCTTTCAAACA TTTTATAAGTAATGTTGTATCTATTTCATGCTTACT [SEQ ID NO:59]</p>
CDH26	<p>Cadherin-like protein 26 [Precursor] 232306 at: GGGAATCACTATTCAGGGAATTTTCCCTTTGCTCTCTTTTCCCTCTCTTAAAGAAAAA</p>

	<p>TTACCTTCTAGTCTAGGATGAGGACACACTATTAGTTTGAATTAATGCTTGTGATATTC TCAGATCAGCCATCTTGAACCAAGCAAAACCACAGGTTACACTTCTTAAATTTGATT TGTGATATTTCTAGAGAACTTGAATTAATTTGTTATTTCTAGCTTCCATCGCAGC CTAGCTTGTAGGGTAAATGAATATAAACCATAGATTACCCAGCCACTTGGGAACAGCA GGTAATACTGAAGAAAAATAAAAAATAGATTTTGAACACGTTANNANANNNTATGATTA TGATTCGTGTTCCATTAAAGGGAACACTTAGCTTAATAGAGAAAAATTTTCTATAACAATTG TGTAGTCAGT</p> <p>[SEQ ID NO:60]</p> <p>Gelsolin [Precursor]</p> <p>20696 s. at:</p> <p>TGCTCTGGACACCTGGGACCAGGCTTTTGCTGGTTGGAAAGGATTCTCAAGAAGAAG AAAAGACAGAAGCCTTGACTTCTGCTAAGCGGTACATCGAGCGGACCAGCCAAATCGGG ATCGGGGACGCCATCACCGTGTGAAGCAAGGCTTTGAGCCCTCCCTCTTTGTGGCT GGTTCCTTGGCTGGGATGATTACTGTGCTGTGACCCCTTGGACAGGCCCATGGCTG AGCTGGCTGCTGAGAGGGGCAAGGCCCAACCATGTCAACCGGTACGTGCTTTTGGAAC TGTCTTCCCTCAAGAGGCCCTTAGAGCGAGCAGCAGCTCTGCTATGAGTGTGTGT</p> <p>[SEQ ID NO:61]</p>
C20RF32	<p>226751 at:</p> <p>ACTTTTGACCACCTTGCTGACTGGAGTTTCAGTGGCCCTGGCAGGCTTGTCTGCTCTTGAC ATTCCACTGACTAACTTTGGTGTGTTNGTTTCCAAGTTAAGTGATTCCTCTCTTTTNGT TCAATGTTAAATTTAAATAATAACAAATGTGTATGGGTCTCCCATGTGTAATATGGTAACA TGTAAATGTCAGTGTTCGACGTTTCAAGCAGGCTTTGTGAAAAATGTAATACAAACAG CAGTGAATGGGACTCAATGTTGTGCTTCTCTATAACAGCTCCGCTTTTCAGGGAAGGA TGGTAACAACTAGAGAGCAATAATATGACGTATTTAATACGTATTAACAACTCTTTTAAAG TAGCTTAAAGGTATGTGCAATGGCTAGCCCTAGTAGAAATGGGGGAAAGCATTTGCTGTG GACCATTTGTTAAAGTGACAGGAGTTGTAGGGTTACCCCTTTTGACAAAGCTTCCATAGTCTT CAGACACGCACCAATTGATGGCATCCCT</p> <p>[SEQ ID NO:62]</p>
TRACH2000196 (TMEM71)	<p>238429 at:</p> <p>CTAACTAAATACCAACCTGACAACTTGAATAACAATAAATGCAATTTGTACATAAAATATN ATGCTGCAAAAGTTNGTCAATTCACCTCAGTGGAGTGTGATATTAGGTGGTNACCGTA</p>

	<p>GATGATGGTTTATATGANAANTGGACAGGAAAGAGCANTTCTCGAAAGTTATANTCTTT TGAACACGTTCTAAACCAAGTNTTNAATCTCTTGGGGCTCGTAATTACCTTTCACCTT AATGCTACTTAAAGATAACACAGAAAATGCTTGGAGCAAAAATATAGGCAAAACAC CAATGCGCTTTCAAAATGCAATGAAATGGTGCAGTTGTACCTTGGAGCTTGTACTCAAGGG CTGTAGATGTTCTCTCCACCCACACTTGGTGGTTCACAAAGCAAAATATGGCC TGAAATGCAAAATTTGTTCTATGTGATACCTCTGAGTAAAAATCTATACATGCGAGAAAT TGCTTTGCTCGAAAT</p> <p>[SEQ ID NO:63]</p> <p>DnaI homolog subfamily C member 12</p> <p>218976_at: CCCAAGCCCTAGAAAGTCAGTCTCCCGCAAAATTCAGATTCTTCAGGTTTGCAGAT GTGAATGGTTGGCACCCTTCTCCGCTGGTCCAAAGGATGCTCCCTCAGAACTCCTGAGG AAGTTCAAGAACTATGAAATATGAAATATCTGCTTCAAAAAATGAGGAAGAGCAAGAC TGTCCCTATGCTGCAACATGCAGTCTTGTATGCTTAAAAATGTCATGTTATGT CATGCTGTGAATGCTGAGTACTAATGATTCCTCCATCCTTGAAATCAGTTCATCAAT GCTTTTAAATAGAAAAATTCAGAAAGTGAATTTCTTCCAAATATTTGAAATAAATTAAG CTCTAGATACAGATGAGTGTATTAATGCTTTTCTCTATTAATCTACTTATAGAAA TCCATTAAGCAATCTCTGTACAGTGTATTTAAATATTTCAATGACATACCTGTGATCT CTATTAGTGTGAGTGTACAAAAATGTTTTCTTACCCTTGACTTACAATGAAAATGTGAA ATTACTGTCTGAACCCCGT</p> <p>[SEQ ID NO:64]</p> <p>Regulator of G-protein signaling 13</p>
DNAJC12	
RGS13**	<p>210258_at: ACAGCAAGCCATGTAGTTCAATTAATATATAAGGAAAAGGAAGGCTTCTTCTCATGATA CAAGCATTAAGTTTTACTGTAGTCAATTAATGGATATTTCCCTTGTAAATAAAA TTTTGTGTCATAATTTACAAATTAGTTCTTAAAAATTTGTGTATATGAATGTGTTC TAGCATGAATGTCTATAGAGTACTCAAAATAACTTGAATTTATAGCAAAATGCTACTCA CAGTACAATCAATGTATATACCATGAGAAAATCAAAAAGGTGTTCTCAGACATTT TATCTAAAAATTTCCCTACTATATGTGTCATTAACAACTCTTTATCACCATGTATCTT CTACGTGTAACACATTTCTGATGATTTTTAACAAAAATATATGAATTTCTTCATTTCG</p>

SLC18A2**	<p>TCTTGCACTACATGCTATAANGGATATAAAATGTGGTTCTCTATATTTTGAGATGTTTT TTCCTACAA'GTGAACCA'CGTGATCTGG [SEQ ID NO:65]</p> <p>Solute carrier family 18 member 2</p> <p>205857 at: CTGCTACTTTGGAAAGATGGCTCTGGAGGAAACTCTATATGGCTAAAAAGGCAGGCTAGT TTCCTACTCTACAGGGTAGAGCTTAAAAAGAACGCTACAAATGGTTNICTNN AGGTTTNCNGGTTCTCCCTGCCCAATNCNATATACTTTANTGCNNTTTATTTTTC CTTACGGNCTCTGTGCTTTCTGCAAGAGGCTGGCAAGGTATGCTGCTGTGGTC CCNTGGGATAAGATAAAATATAAACTTCAGACTGTTTGGAGCAAAAGATAG CTTGACTTGGGAAAAAATCTAAGTCTTTTATATGACTAATATCTTGGTTAGCAA GACTGGAAGAGGTGTTTTTAAATGTACATACACGAGCAAGAACATACAGCTCTCT GAACATTTATTTTGAACAGAGGTGGTTTTTATGTTTGGACCTGGTAAATACAGATACAA AAACTTTAATGAGGTAGCAATGAATATCAACTGTTTGACTGCTAAGTATCTGTCCAT ATTTAGCAAG [SEQ ID NO:66]</p>
SERPINB10	<p>Serpin peptidase inhibitor clade B (Ovalbumin) member 10</p> <p>214539 at: TACTACAAAGCCGTGACCTCAGCCTGCTTATACTACTGCCAGAAAGACATTAAATGGGCTG GAACAGCTGGAAGAGGCCATCACTATGAGAAAGCTGAATGAGTGGACCATGCGACATG ATGGAGTTGTATGAAGTGACGTACACCTTCCAAAGTTCAAGCTGGAAGACAGATATGAT CTCAAGTCAACCCCTGAGCAGTATGGGATGAGTGATGCCTCAGCCAAAGCAAGCTGAT TTCTCAGGAATGCTTCAGCAAGAAACCTATTTTGTCCAAATGTTTTCATAGGCTTTT GTGAAATAAATGAACAAGGTACTGAAGCTGCAAGCTGGCAGTGGGAGTGAGATAGATA CGAANTAGAGTCCCATCCATTGAATCAATGCAATACCCCATCTCTCTTCATCAGG CACAAATANAACCAACACCATCTCTTTTATGGAAGATTATGCTCCCCCTAATC [SEQ ID NO:67]</p>
SH3RF2	<p>SH3 domain-containing RING finger protein 2</p> <p>243582 at:</p>

	<p>GATTC1GTGGTAGACTCAGTGTCTTCAGAGTCCAGAGCTTGAC1TGGGT1AGTGGCCTTA ATGAAGTCTAAATTTGCTCTTACCGGAGACTGATCAGAAAGCAAAAGGGGAAAGG GGGCTAGAGTCCATCGCACTTTTACATCAGACAAGAGGAGGACTGTGCCAGAAATCT GTGCATGAACAACCATCTGCTCTTCATGCGGAGGGGTCACCGTGTGAACGTGCAGAG ATTACTCGAGCCCTCTTTGGCAAAAATATGCAATCTTCCAGCTGTA [SEQ ID NO:68]</p>
FCER1B**	<p>Fe epsilon1R/beta</p> <p>207496 at:</p> <p>TAAATCACATCACTTCATGGCATGGATGTTTCACATACAGAGCTTTAAACCTGGTTTACCA GGACCTCTAGGAGTGGATCCAAATCTATATCTTTACAGTTGTATAGTATATGATATCTCTT TTATTTCACTCAATTTATATTTTTCATCATTTGACTACATATTTCTTATACACAACACAAA TTTATGAATTTTTCACAGATCATCTGAGAGTTGCCCACTTACCTGCTTTTATAG TACGCCCACTCAGGCACACAGAGCAAAATGCTGGGGTTCTCTCACACTATCACTGC CCCAATTTGCTCTTCTAAATTTCAACTTCAATGTCACTCTCCATGAAGACCACTGAAT GAACACCTTTTCATCCAGCCTTAATTTCTTGCTCCATAACTACTCTATCCACGATGCAG TATTGTATCATTAATTAATAGTGTGCTGTGACCTCTTATGTATTTCTCAATTAACCTGTA TTTGTGCAATAAAATTGGAAATAATGTAACCTGATTTCTTATCTGTGTTTGTGGCATGC AAGA1 [SEQ ID NO:69]</p>
RUNX2	<p>Runt-related transcription factor 2</p> <p>232231 at:</p> <p>AAGACACTCTTCCAAAACCTTGAAATTTGTTGTTTTAGAAAACGAATGCATTTAAAAATA TTTCTATGTGAGAAATTTTATAGATGTGTTTACTTTCATGTTTACAAATAACTGTTCG TTTITATGCACTTGTGAATAATATACGCCAAAACCAATACATTAATAATTTCTTAG GTAATCTGAATAAAATCCATTTCTTTGGATATGCTTTTACCACTTAGGTTTCTGTGG AACAATAATATTGTAGCAATTTGTGTAATAACAGCTTTCATTTTATTTTTCCAAT GCTATTGCCCAAGAAATGCTTTCATGCACATATTGTAAAAATCCCGCTTTGTGCCACAG GTCAATGTTGTGGATGATTTACTCTTAACTTCAAGGGGACTAATTGTATTTGTAATGTTGC [SEQ ID NO:70]</p>
PTGS1	<p>Prostaglandin-endoperoxide synthase 1</p>

Example 8 – Relationship of “IL-13 high” and “IL-13 low” subphenotypes of asthma to clinical features

[00142] The asthmatic subjects were further analyzed with respect to additional demographic characteristics and clinical features as those described in Example 7. The results are shown in Table 5 and Figs. 5 and 6. Although subjects with “IL-13 high” asthma subphenotype could not be distinguished from subjects with “IL-13 low” asthma subphenotype based on demographic characteristics, lung function, or bronchodilator responsiveness (delta FEV₁ with albuterol) (Table 5, Figures 5A-B), these groups differed significantly with respect to degree of airway hyper-responsiveness (AHR, PC₂₀ to methacholine, defined as the minimal concentration of methacholine required to induce a 20% decrease in expiratory airflow, Figure 5C). This difference in AHR was apparent despite inclusion criteria that required all asthmatics to have significant AHR (all asthmatics < 8 mg/ml, all healthy controls > 20 mg/ml).

Table 5. Subject characteristics by asthma phenotype

	Healthy Control	Asthma		p-value low vs. high
		IL13 Signature Low	IL-13 Signature High	
Sample size	28	20	22	-
Age	36±9	36±11	37±12	0.98
Gender, M:F (% F)	12:16 (56)	6:14 (70)	11:11 (50)	0.19
Ethnicity				
Caucasian	20	9	9	0.98
African-American	0	4	4	
Hispanic	3	5	6	
Asian/Pacific Islander	5	2	3	
FEV ₁ , % predicted	107 (13)	89 (10)	85 (13)	0.85
ΔFEV ₁ with albuterol (%)	2.7±3.4%	9.7±7.4%	12.5±9.8	0.51

of baseline)				
Methacholine PC ₂₀	64 (22-64)	0.93 (0.06-7.3)	0.27 (0.05-1.9)	<0.001
IgE, IU/ml	27 (3-287)	125 (19-1194)	244 (32-2627)	0.031
	N=26			
Blood eosinophils, ×10 ⁹ /L	0.10±0.07	0.23±0.21	0.37±0.22	0.027
BAL eosinophil %	0.26±0.29	0.42±0.46	1.9±1.9	0.001
	N=22	N=16	N=20	
RBM thickness, μm	4.34±1.11	4.67±0.99	5.91±1.72	0.014
	N=22	N=19	N=19	
ΔFEV ₁ with fluticasone at 4 weeks, L	N/A	0.03±0.12	0.35±0.2	0.004
		N=6	N=10	
ΔFEV ₁ with fluticasone 8 weeks, L	N/A	0.04±0.12	0.25±0.23	0.05
		N=6	N=10	

For normally distributed data, values are presented as mean±standard deviation and student's t-test performed; for non-normally distributed data, values are presented as median (range) and wilcoxon rank sum test performed. In case of missing data, number of subjects for whom data exist noted. P-values relative to healthy control also depicted in Figs. 5 and 6. PC₂₀ denotes the provocative concentration required to cause a 20% decline in FEV₁; BAL, bronchoalveolar lavage; RBM, reticular basement membrane.

[00143] To determine whether the IL-13 subphenotype of an individual subject was correlated with measures of allergic inflammation, we examined the results of skin prick tests (SPT) to a panel of 12 aeroallergens (Table 6), levels of serum IgE, peripheral blood eosinophil counts, and eosinophil percentages in bronchoalveolar lavage fluid (BAL). The results are shown in Figures 6A-D and 7A-B. Both IL-13 high and low asthma subphenotypes had increased SPT sensitivity to aeroallergens as compared to healthy controls (Fig. 6A), although the IL-13 low asthma subphenotype tended to have fewer positive skin tests than the IL-13 high asthma subphenotype and to be sensitized less frequently to aeroallergens such as dog and house dust mite (Fig. 7A). Subjects with IL-13 high asthma subphenotype had higher serum IgE levels and higher peripheral blood eosinophil counts than subjects with IL-13 low asthma subphenotype, although IL-13 low asthma subphenotype

differed from healthy controls with respect to these features of allergic inflammation (Figs. 6B-C). In addition, subjects with IL-13 high asthma subphenotype had increased eosinophil numbers in the lung as assessed by BAL (Fig. 6D), whereas IL-13 low asthmatics did not differ from healthy controls in BAL eosinophil percentage. These data demonstrate enrichment for AHR, IgE levels, and eosinophilic inflammation in subjects with the IL-13 high asthma subphenotype, but SPT sensitivity to aeroallergens was not restricted to this subgroup. Thus, it is likely that alternate non-Th2 mechanisms for sensitization to aeroallergens operate in subjects with the IL-13 low asthma subphenotype.

Table 6. Allergen skin prick test panel

Allergen		
D. farinae	Cladosporium herbarum	West Oak mix
D. pteronyssius	Cat	Grass mix/Bermuda/Johnson
American Cockroach	Dog	Histamine [10mg/ml] (positive control)
Alternaria tenuis	Plantain-Sorrel mix	50% Glycerin (negative control)
Aspergillus mix	Short Ragweed	

[00144] To determine whether the subphenotype of IL-13 high asthma is durable or a transient manifestation of Th2-driven inflammation due to recent exposure to allergen, we measured pathological changes in bronchial biopsies from the same subjects. We and others have previously demonstrated that asthma is associated with pathological changes known as airway remodeling and which reflect either longstanding inflammation or the effects of injury and repair over time [28, 29]. Two specific remodeling outcomes in asthma are airway fibrosis, manifest as thickening of the sub-epithelial reticular basement membrane (RBM) [30, 31] and increased mucin stores in the airway epithelium [32]. We found that RBM thickness was greater in subjects with IL-13 high asthma subphenotype than in IL-13 low asthma subphenotype or healthy controls and that RBM thickness was normal in the IL-13 low subphenotype of asthma (Fig. 6E). In addition, although we observed a trend toward increased epithelial mucin stores in both subphenotypes of subjects with asthma, this increase was significant only in subjects with IL-13 high asthma subphenotype (Fig. 8A). Although these differences in total mucin stores were modest, qPCR revealed a striking difference in the expression levels of the major gel-forming mucins in airway epithelial cells in IL-13 high asthma subphenotype as compared to both IL-13 low asthma subphenotype and healthy controls (Figs. 8B-D). Specifically, IL-13 high asthma subphenotype was distinguished from IL-13 low asthma subphenotype and healthy controls by induction of MUC5AC and MUC2

expression and repression of MUC5B expression. This alteration in the expression of specific mucin genes in IL-13 high asthma subphenotype is most evident in the ratio of MUC5AC to MUC5B expression (Fig. 6F). Without being bound by theory, we speculate that concomitant induction and repression of specific gel-forming mucins may explain the relatively modest increase in epithelial mucin stores in IL-13 high asthma subphenotype compared to IL-13 low asthma subphenotype and healthy controls. Taken together, these findings indicate that IL-13 high asthma subphenotype is associated with remodeling changes in the airway that identify this subphenotype as durable over time. These results also demonstrate the importance of the IL-13 pathway to airway remodeling in human subjects.

[00145] Alveolar macrophages may modulate allergic airway inflammation in asthma as a source of IL-13 [54] and leukotrienes or eicosanoid lipids [55, 56] or through “alternative activation” under the influence of IL-13 [57]. To determine whether alveolar macrophages from subjects with “IL-13 high” asthma manifest any of these findings, we measured the expression of relevant genes using qPCR in 14 subjects with asthma and 15 healthy controls (Table 7). We found no evidence for induction of Th2 cytokines or of alternative activation markers in asthma generally or in the “IL-13 high” subgroup specifically. Levels of expression of IL-13 were below the limit of detection (cycle threshold >40) in 26 of the 29 subjects, and IL-4 was below the limit of detection in 20 of the 29 subjects (no differences between the three groups for either cytokine, all $p>0.35$). All other genes were within the limit of detection across samples. In these analyses we found increased expression of 15-lipoxygenase in “IL-13 high” asthma (Figure 10, Table 8), consistent with prior findings of increased 15-lipoxygenase products in the airways in severe eosinophilic asthma [56]. We also found an increase in expression of TNF α that was limited to the “IL-13 high” subgroup (Figure 10, Table 8).

[00146] Only a subset of asthmatics manifests improvement in lung function when treated with inhaled corticosteroids (ICS) [33]. To identify gene expression markers of corticosteroid responsiveness, we measured FEV₁ in a subset of our subjects with asthma during an 8-week randomized controlled trial of inhaled fluticasone or placebo as previously reported[8]. When we re-analyzed that data while stratifying subjects by IL-13 subphenotype, we found that improvements in FEV₁ were limited to those with the IL-13 high subphenotype. Specifically, the subjects with the IL-13 high asthma subphenotype who were treated with inhaled fluticasone had significant improvements in FEV₁ at both 4 and 8 weeks as compared to subjects treated with placebo, whereas subjects with IL-13 low asthma subphenotype did not (Fig. 9A). These improvements in FEV₁ in the IL-13 high group were lost after a one week

run out period off drug. There was no significant change in FEV₁ in response to placebo at any timepoint in either group (data not shown, N=5 “IL-13 high,” N=6 “IL-13 low”). As described previously [8], we performed a second bronchoscopy one week after the initiation of treatment and analyzed gene expression in bronchial epithelium by microarray as at baseline. In re-analyses of these data, while stratifying subjects by IL-13 subphenotype, subjects with IL-13 high asthma at baseline continued to exhibit a strong IL-13 subphenotype after one week of placebo treatment demonstrating the short-term stability of this subphenotype in the absence of therapy. However, after one week of fluticasone treatment, subjects with IL-13 high asthma clustered with subjects who were IL-13 low at baseline, regardless of treatment (Fig. 9B). Thus, the phenotypic classification of asthma based on the IL-13 signature described herein predicts response to ICS. These data suggest that the global benefit of ICS treatment for asthma is accounted for by the IL-13 high subphenotype.

[00147] Our results provide new insights into molecular mechanisms that underlie clinical heterogeneity in asthma. Basic research previously established IL-13 and related Th2 cytokines as central regulators of allergic inflammation and many of the pathophysiologic changes associated with asthma [35, 36]. Here, using gene expression profiling, we have identified an “IL-13 high” subphenotype in patients with asthma. Using rigorous clinical criteria and methacholine challenge testing, we found that that this subphenotype comprises only ~50% of patients who are diagnosed with asthma. This “IL-13 high” subphenotype also displayed increased levels of IL-5 expression and showed certain distinguishing clinical characteristics including enhanced airway hyper-responsiveness, increased serum IgE levels and eosinophilic inflammation, subepithelial fibrosis, and altered expression of gel-forming mucins compared to an “IL-13 low” subphenotype and healthy controls.

[00148] Our work challenges certain current concepts of asthma pathogenesis by showing that a gene signature for IL-13 driven inflammation in airway epithelial cells is prominent in only half of asthmatics; non-IL-13 driven mechanisms must therefore operate in the remaining half. The findings discussed herein lead us to propose that asthma can be divided into various molecular subphenotypes such as “IL-13 high” and “IL-13 low” subphenotypes referred to herein. We validated the IL-13 high/IL-13 low classification scheme through confirmatory analyses of gene expression in bronchial biopsies, analysis of reproducibility on repeat examination, and comprehensive characterization of the distinct clinical, inflammatory, pathological and treatment-related characteristics of these two molecular subphenotypes of asthma. These findings provide a mechanistic framework for the emerging clinical observation that asthma is a complex and heterogeneous disease [58].

[00149] Molecular phenotyping of asthma based on Th2 inflammation has important therapeutic implications. First, airway obstruction in the “IL-13 high” subphenotype improves with inhaled steroids whereas the “IL-13 low” subphenotype shows little to no improvement. The Th2 markers that we have identified can be used to guide the development of clinical tests for steroid-responsiveness by providing surrogate markers of a steroid-responsive phenotype. Second, blockade of IL-13 and related Th2 cytokines is under active clinical development as a therapeutic strategy in asthma [34]. Our data suggest that clinical response to these therapies may be limited to the specific subphenotype of patients with “IL-13 high” asthma. Thus, markers of this molecular phenotype have direct application in clinical trials.

[00150] Prior studies using induced sputum analyses suggested that “eosinophilic asthma” is a distinct cellular phenotype of asthma, but molecular mechanisms underlying this cellular phenotype have been undefined. Our data suggest that IL-13 driven inflammation is a molecular mechanism underlying “eosinophilic asthma” [37] because of the airway eosinophilia that we demonstrated in “IL-13 high asthma.” In addition, we demonstrated that both “eosinophilic asthma” and “IL-13 high” asthma are characterized by subepithelial fibrosis [38, 39], ALOX15 production by alveolar macrophages [55] and lung function responses to inhaled corticosteroids [40, 41]. In addition to these recognized features of eosinophilic asthma, we have identified further clinical features of “IL-13 high” asthma, including altered airway mucin gene expression and induction of TNF α , a mediator which is not considered a Th2-cytokine but which has been previously associated with severe asthma [59]. We speculate that these features will also be found in eosinophilic asthma. In addition, it is likely that IL-5 is a major contributor to the airway and systemic eosinophilia we observe in “IL-13 high” asthma, because we found that IL-5 expression is significantly co-regulated with IL-13 expression (Fig. 1E). IL-5 is a major stimulus of eosinophil differentiation, recruitment, activation, and survival [60], but IL-13 can strongly induce the expression of eosinophil chemoattractants such as CCL11, CCL22, and CCL26 in the airway [61] and may thus work cooperatively with IL-5 to promote eosinophil infiltration, activation, and survival in the airways. Residual IL-13 activity may therefore explain the incomplete tissue depletion of eosinophils observed in clinical trials of IL-5 blockade in asthma [62, 63].

[00151] In addition, these data reveal that a significant percentage of patients with asthma have an “IL-13 low” phenotype which manifests such clinical features of asthma as airway obstruction, airway hyper-responsiveness and bronchodilator reversibility despite a paucity of

Th2-driven inflammation. The causes of “IL-13 low” asthma remain obscure, but possibilities include neutrophilic inflammation [37], IL-17 driven inflammation [42], intrinsic defects in barrier function [43] and chronic sub-clinical infection by atypical intracellular bacteria [44].

Table 7. Genes used in alveolar macrophage qPCR

Symbol	Name	Category	Entrez Gene ID
IL13	interleukin 13	Th2 cytokine	3596
IL4	interleukin 4	Th2 cytokine	3565
ARG1	arginase, liver	Alternative activation marker	383
MRC1	mannose receptor, C type1	Alternative activation marker	4360
MRC2	mannose receptor, C type2	Alternative activation marker	9902
IL1RN	interleukin 1 receptor antagonist	Alternative activation marker	3557
CCL17	T cell-directed CC chemokine	Alternative activation marker	6361
CCL22	macrophage derived chemokine	Alternative activation marker	6367
TNF α	tumor necrosis factor	Classical activation marker	7124
IL1 β	interleukin 1, beta	Classical activation marker	3553
CCL20	macrophage inflammatory protein 3 alpha	Classical activation marker	6364
ALOX15	arachidonate 15-lipoxygenase	Leukotriene pathway	246
ALOX5	arachidonate 5-lipoxygenase	Leukotriene pathway	240
ALOX5AP	arachidonate 5-lipoxygenase-activating protein	Leukotriene pathway	241
LTA4H	leukotriene A4 hydrolase	Leukotriene pathway	4048
LTC4S	leukotriene C4 synthase	Leukotriene pathway	4056

Table 8. Alveolar macrophage gene expression by qPCR

Gene	Normalized Gene Copy Number			P-values		
	Control N=15	IL-13 Low N=5	IL-13 High N=9	IL-13 Low vs. control	IL-13 High vs. control	IL-13 High vs IL-13 Low
IL13	---	---	---	---	---	---
IL4	---	---	---	---	---	---
ARG1	16,707 \pm 49,889	13,188 \pm 29,285	177 \pm 349	0.99	0.68	0.91

Gene	Normalized Gene Copy Number			P-values		
	Control N=15	IL-13 Low N=5	IL-13 High N=9	IL-13 Low vs. control	IL-13 High vs. control	IL-13 High vs IL-13 Low
MRC1	4,729,405± 2,343,659	4,281,358± 2,235,805	5,575,399± 2,211,337	0.98	0.77	0.69
MRC2	323,199± 949,034	318,115± 704,525	1,627±929	1.00	0.68	0.84
IL1RN	1,217,545± 2,179,904	1,629,394± 2,679,369	477,775± 147,251	0.97	0.75	0.64
CCL17	200±457	421±867	42±44	0.76	0.82	0.42
CCL22	61,812± 163,171	53,105± 113,545	4,306±5,750	0.99	0.65	0.88
TNF α	75,044± 41,433	75,941± 43,938	130,385± 47,351	1.00	0.017 *	0.10
IL1 β	102,121± 37,416	107,456± 20,675	111,181± 25,317	0.98	0.88	0.99
CCL20	16,033± 9,224	16,826± 7,375	16,231± 5,003	0.99	1.00	0.99
ALOX15	18,741± 19,420	24,167± 19,036	142,494± 188,198	1.00	0.03 *	0.16
ALOX5	10,655,887± 2,754,206	1,1308,968± 2,851,849	11,033,153± 1,397,415	0.94	0.98	0.99
ALOX5AP	13,940,937± 3,209,466	12,710,464± 2,864,216	12,877,643± 2,812,301	0.83	0.80	1.00
LTA4H	8,532,533± 1,944,551	8,455,408± 1,191,877	7,859,076± 1,647,800	1.00	0.75	0.91
LTC4S	4,959±3,748	5,445±3,189	9,086±4,988	0.99	0.07	0.33

Levels of expression of IL-13 were below the limit of detection (cycle threshold >40) in 26 of the 29 subjects, and IL-4 was below the limit of detection in 20 of the 29 subjects (no differences between the three groups for either cytokine, all $p>0.35$). All other genes were within the limit of detection across samples.

Example 9 – Relationship of “IL-13 high” and “IL-13 low” subphenotypes of asthma to serum protein biomarkers

[00152] Further microarray analysis led us to identify from the set of genes and probes listed in Table 4, a set of 35 probes representing 28 genes whose expression was co-regulated with periostin in individual subjects below a threshold false discovery rate (FDR) q-value of 0.05. These genes and probes and associated data are presented in Table 9. Hierarchical cluster analysis of all subjects, including healthy controls and asthmatics, based on expression

levels of those probes confirmed and further defined the two major clusters described above of (1) a cluster with high expression levels of periostin and co-regulated genes and (2) a cluster with low expression levels of periostin and co-regulated genes (Figure 11). Mast cell genes include RGS13, TPSG1, TPSAB1, FCER1B, CPA3 and SLC18A2. Eosinophil genes include P2RY14 and ALOX15.

[00153] The cluster with high expression of periostin and co-regulated genes comprised 23 asthmatic subjects and 1 healthy control (Figure 11, cluster 1, indicated in red) whereas the cluster with low expression of periostin and co-regulated genes comprised the remaining 19 asthmatics interspersed with 26 of the healthy controls (Figure 11, cluster 2, indicated in green). In Example 8, we described clustering of subjects in this dataset based on the microarray-determined expression levels of three of these probes: 210809_s_at (periostin), 210107_at (CLCA1), and 204614_at (serpinB2). The three-probe signature described in Example 8 correlates well with this full 35-probe signature, differing for seven asthmatics and one healthy control (discrepant calls indicated in Figure 11 with *).

Table 9. IL-13 gene signature genes and exemplary probes.

Microarray signal intensity											
Probe	Gene Name	fold high vs. low	IL13low vs high Pval	IL13low vs high qval	IL13low vs. HC Pval	IL13low vs. HC qval	Healthy Mean	IL13 Low mean	IL13 high Mean	fold high vs. HC	fold low vs. HC
206224 at	CS11*	15.46	2.33E-06	0.004	0.87	0.95	8.76	17.86	276.18	31.54	2.04
208555 x at	CS12*	12.69	3.02E-07	0.001	0.86	0.95	5.13	8.95	113.65	22.16	1.75
206994 at	CS14*	10.87	1.29E-06	0.002	0.71	0.94	10.38	48.27	524.78	50.56	4.65
210107 at	CLCA1*	9.05	2.28E-07	0.001	0.71	0.94	29.61	74.20	671.32	22.67	2.51
223710 at	CC126*	8.83	4.60E-05	0.030	0.74	0.95	6.33	3.79	33.50	5.29	0.60
1555778 a at	POSTN*	7.21	9.58E-09	4.90E-05	0.88	0.95	14.93	17.89	129.07	8.65	1.20
210809 s at	POSTN*	5.31	3.90E-13	1.93E-08	0.60	0.94	260.24	372.07	1976.14	7.59	1.43
204919 at	PRR4*	5.14	1.75E-06	0.003	0.49	0.94	37.33	97.62	502.21	13.45	2.62
207741 x at	TPSD1**	4.40	1.90E-05	0.02	0.69	0.94	9.86	14.84	65.30	6.62	1.51
204614 at	SERPINB2*	3.72	2.84E-07	0.001	0.35	0.92	97.43	196.39	729.95	7.49	2.02
216485 s at	TPSG1**	3.65	8.94E-09	4.90E-05	0.61	0.94	10.04	13.18	48.06	4.79	1.31
210037 s at	INOS*	3.43	2.53E-07	0.001	0.91	0.95	6.39	6.07	20.86	3.26	0.95
210258 at	RGST3**	3.19	1.88E-07	0.001	0.08	0.87	8.75	4.97	15.84	1.81	0.57
2011884 at	CEACAM5	2.89	1.30E-08	5.82E-05	0.54	0.94	426.04	535.38	1548.02	3.63	1.26
216474 x at	TPSD1**	2.88	7.64E-10	1.26E-05	0.29	0.91	46.63	69.06	199.21	4.27	1.48
206637 at	GPR105***	2.75	3.19E-08	0.0001	0.45	0.94	40.90	53.89	148.29	3.63	1.32
205624 at	CPIA3**	2.58	3.91E-09	2.76E-05	0.25	0.90	99.69	145.25	374.66	3.76	1.48
226751 at	C2ORF32*	2.55	4.58E-08	0.0002	0.52	0.94	35.39	29.78	76.07	2.15	0.84
205683 x at	TPSD1**	2.54	3.57E-10	8.82E-06	0.26	0.90	53.99	74.71	189.54	3.51	1.38
210084 x at	TPSD1**	2.45	2.01E-09	1.99E-05	0.23	0.90	48.91	69.02	189.00	3.46	1.41
225316 at	MFS2	2.44	2.54E-06	0.004	0.88	0.95	29.03	27.72	67.73	2.33	0.95
207134 x at	TPSD1**	2.37	9.91E-09	4.90E-05	0.12	0.88	50.28	80.13	189.76	3.77	1.59
232306 at	CDH26	2.31	3.31E-07	0.001	0.23	0.90	223.92	330.01	763.78	3.41	1.47
215382 x at	TPSD1**	2.22	1.89E-07	0.001	0.40	0.93	38.96	48.85	108.32	2.78	1.25
200996 s at	GSN	2.08	3.45E-06	0.005	0.95	0.95	246.87	243.40	506.71	2.05	0.99
217023 x at	TPSD1**	2.02	8.11E-08	0.0003	0.24	0.90	53.13	68.70	136.67	2.61	1.29
205657 at	SLC18A2**	1.88	1.29E-06	0.002	0.39	0.93	84.08	100.24	188.75	2.24	1.19
214539 at	SERPINB10	1.88	1.20E-05	0.012	0.54	0.94	42.37	37.53	70.62	1.67	0.89
207496 at	FCER1B**	1.82	4.18E-06	0.005	0.52	0.91	37.55	33.51	61.04	1.63	0.89
238429 at	TMEM71	1.82	3.45E-05	0.024	0.51	0.94	36.79	42.73	77.72	2.11	1.16

Probe	Gene Name	fold high vs. low	IL13low vs high Pval	IL13low vs high qval	IL13low vs. HC Pval	IL13low vs. HC qval	Healthy Mean	IL13 Low mean	IL13 high Mean	fold high vs. HC	fold low vs. HC
243582 at	SH3RF2	1.80	1.48E-07	0.0005	0.36	0.92	82.64	96.39	173.86	2.10	1.17
218976 at	DNAJC12	1.79	9.57E-05	0.044	0.59	0.94	48.54	43.54	77.72	1.60	0.90
238669 at	PTGS1	1.56	2.04E-06	0.003	0.70	0.94	82.69	86.79	135.37	1.60	1.05
207328 at	ALOX15***	1.56	7.87E-06	0.003	0.36	0.92	812.57	919.86	1424.90	1.75	1.13
232231 at	RUNX2	1.56	4.99E-05	0.032	0.28	0.91	288.42	335.71	502.48	1.74	1.16

Probes are ranked in order of fold change in "IL-13 high" vs. "IL-13 low" asthmatics (third column from left); probes with a 2.5 fold or greater enrichment in "IL-13 high" asthma are shown with bolded gene names.

Probes corresponding to periostin (POSTN) and CEACAM5 are shaded.

Non mast cell genes > 3-fold upregulated in "IL-13 high" vs. "IL-13 low" asthma are indicated with a single asterisk (*).

Mast cell-specific genes are indicated with a double asterisk (**). (Note that based on clustering pattern, C2ORF32 signal is likely mast cell-derived).

Eosinophil-specific genes are indicated with a triple asterisk (***).

[00154] Using the three-gene (periostin, CLCA1, and serpinB2) IL-13 signature, we showed in Example 8 that systemic markers of allergic inflammation including serum IgE and peripheral blood eosinophil levels were significantly elevated in “IL-13 high” subphenotype asthmatics relative to “IL-13 low” subphenotype asthmatics. However, there was significant overlap between the asthmatic groups for each of these metrics taken individually. In addition, neither serum IgE or peripheral blood eosinophil levels alone constitutes a non-invasive metric for predicting the airway IL-13 signature and associated “IL-13 high” or “IL-13 low” asthma subphenotype with simultaneous high sensitivity and specificity.

[00155] To determine whether the intersection of IgE and peripheral blood eosinophil levels could predict patterns of airway inflammation with greater accuracy than either metric alone, we evaluated serum IgE and peripheral blood eosinophil counts together versus airway IL-13 signature status. We found that, across the 42 asthmatics, serum IgE and peripheral blood eosinophil counts were correlated, albeit weakly (Figure 4; data shown for the IL-4/13 signature; similar results were obtained for the IL-13 signature [see Table 10]). For the IL-13 signature, all of the “IL-13 high” asthmatics had eosinophil counts greater than $0.14 \times 10^9/\text{L}$, but many of the “IL-13 low” asthmatics had lower eosinophil counts. All but two of the “IL-13 high” asthmatics had serum IgE levels greater than 100 IU/ml, but many “IL-13 low” asthmatics did not. The two metrics of (1) serum IgE ≥ 100 IU/ml and (2) eosinophil counts $\geq 0.14 \times 10^9/\text{L}$ combined yielded improved sensitivity and specificity for the IL-13 signature in the airway (Table 10). Thus, a composite of two commonly used peripheral blood metrics of allergic inflammation may be an effective noninvasive biomarker for airway IL-13 driven inflammation.

Table 10. Sensitivity, specificity, positive and negative predictive values of IgE and peripheral blood eosinophil metrics for the IL-13 signature.

Positive criteria: serum IgE > 100 IU/ml		IL-13 signature status		Sensitivity: 21/23 = 0.91
		High	Low	
Test Result	+	21	10	PPV: 21/31 = 0.68
	-	2	9	NPV: 9/11 = 0.82
Positive criteria: eosinophils ≥ 0.14x10 ⁹ /L		IL-13 signature status		Sensitivity: 23/23 = 1
		High	Low	
Test Result	+	23	11	PPV: 23/34 = 0.68
	-	0	8	NPV: 8/8 = 1
Positive criteria: IgE > 100 IU/ml AND eosinophils ≥ 0.14x10 ⁹ /L		IL-13 signature status		Sensitivity: 21/23 = 0.91
		High	Low	
Test Result	+	21	5	PPV: 21/26 = 0.81
	-	2	14	NPV: 14/16 = 0.88

[00156] To identify additional systemic (noninvasive) candidate biomarkers of the bronchial epithelial IL-13 signature, we examined the signature for genes encoding extracellular or secreted proteins that might be detectable in peripheral blood. Three candidates of particular interest were CCL26, periostin, and CEACAM5. As CCL26 has been previously described as a Th2 cytokine-induced chemokine in bronchial epithelium [71], we focused on the characterization of periostin and CEACAM5, which have not previously been described as serum biomarkers of Th2 inflammation. CEACAM5 encodes carcinoembryonic antigen (CEA), which is a frequently used prognostic serum biomarker in epithelial-derived cancers. Periostin has also been described in a limited number of studies as a serum biomarker for certain cancers and, intriguingly, was detectable at a level in the range of 10s-100s of ng/ml serum in most subjects, attractive characteristics for a serum marker to be readily detected by immunoassays.

[00157] As shown in Figure 12A-B, Periostin and CEACAM5 are each good individual representatives of the IL-13 signature, exhibiting significantly higher expression in "IL-13 high" asthmatics than in "IL-13 low" asthmatics or healthy controls. There was a strong correlation between microarray expression levels of periostin and CEACAM5 in individual asthmatics (Figure 12C). To confirm these gene expression patterns and determine whether periostin and CEACAM5 expression could be used in an algorithm to distinguish "IL-13 high" asthmatics from "IL-13 low" asthmatics and healthy controls, we analyzed expression levels of the two genes by qPCR in the same bronchial epithelial brushing samples used for

microarray analysis. There was a high degree of concordance between microarray and qPCR values in individual subjects (not shown). We used ordinal logistic regression analysis to generate a predictive model for the microarray-derived 35-probe IL-13 status using qPCR values for periostin and CEACAM5. The model's predictive value was highly significant ($p < 0.0001$) and periostin and CEACAM5 parameter estimates each had a significant effect in the model ($p < 0.02$ for CEACAM5; $p < 0.0001$ for periostin). Receiver operating characteristic (ROC) curve analysis demonstrated perfect predictivity for healthy control and very high sensitivity and specificity for "IL-13 high" and "IL-13 low" asthma (Figure 12D). Taken together, these data show that bronchial epithelial expression levels of periostin and CEACAM5 are good surrogates for the overall IL-13 signature.

[00158] To determine whether elevated levels of soluble periostin and CEA proteins were detectable in peripheral blood, we examined periostin and CEA in sera from 100 asthmatics and 48 healthy controls using immunoassays. In addition, we measured IgE and YKL-40, a serum marker previously described to be elevated in some asthmatics [72], in these same sera. We observed significantly elevated levels of IgE, periostin, CEA, and YKL-40 in asthmatics relative to healthy controls (Figure 13A-D). However, in all cases, there was substantial overlap in serum levels of each biomarker between groups. As shown in Example 8, inhaled corticosteroid (ICS) treatment reduces the bronchial epithelial expression of periostin in asthmatics that have elevated periostin at baseline (*see also* [8]). Of the 100 asthmatics whose serum we examined, 51 were taking inhaled corticosteroids (ICS) and 49 were not. When comparing asthmatics not on ICS and asthmatics on ICS, ICS-treated subjects had significantly lower median serum levels of IgE and CEA, and showed a trend for lower periostin levels, while YKL-40 levels were unchanged (Figure 13E-H). Nevertheless, asthmatics on ICS had higher median serum levels of IgE, periostin, and CEA than healthy controls (Table 13). As shown in Figure 4 and Table 10, 21/23 asthmatics positive for the bronchial epithelial IL-13 signature ("IL-13 high") had serum IgE levels greater than 100 IU/ml, although a proportion of "IL-13 low" asthmatics also had elevated IgE. We found that serum periostin levels trended higher and CEA levels were significantly higher in asthmatics with IgE ≥ 100 IU/ml ($N=68$) than in asthmatics with IgE < 100 IU/ml ($N=32$; Figure 13I-J). However, serum YKL-40 levels were significantly lower in the high IgE group (Figure 13K). As airway expression levels of periostin and CEACAM5 were highly correlated in "IL-13 high" asthmatics, we examined the correlation between serum periostin and CEA across all asthmatics (Figure 13L). We found that serum periostin and CEA levels were significantly correlated with each other across the asthmatic population, and within asthmatics not on ICS

or asthmatics with IgE ≥ 100 IU/ml but not in healthy controls, asthmatics on ICS, or asthmatics with IgE < 100 IU/ml (Table 11). Taken together, these data suggest that periostin and CEA may be serum biomarkers of a bronchial epithelial IL-13 induced gene signature in asthmatics.

Table 11. Correlations between serum biomarkers.

Variable	by Variable	Spearman ρ	P-value
All subjects (Controls, N=48; Asthmatics, N=100)			
YKL40 (ng/ml)	IgE (IU/ml)	0.0140	0.8661
CEA (ng/ml)	IgE (IU/ml)	0.4040	<.0001
CEA (ng/ml)	YKL40 (ng/ml)	0.2935	0.0003
Periostin (ng/ml)	IgE (IU/ml)	0.2259	0.0058
Periostin (ng/ml)	YKL40 (ng/ml)	0.1253	0.1291
Periostin (ng/ml)	CEA (ng/ml)	0.3556	<.0001
Healthy Controls (N=48)			
YKL40 (ng/ml)	IgE (IU/ml)	0.0420	0.7768
CEA (ng/ml)	IgE (IU/ml)	-0.0996	0.5007
CEA (ng/ml)	YKL40 (ng/ml)	0.1914	0.1926
Periostin (ng/ml)	IgE (IU/ml)	-0.2451	0.0931
Periostin (ng/ml)	YKL40 (ng/ml)	0.2246	0.1249
Periostin (ng/ml)	CEA (ng/ml)	0.4495	0.0014
All Asthmatics (N=100)			
YKL40 (ng/ml)	IgE (IU/ml)	-0.2144	0.0322
CEA (ng/ml)	IgE (IU/ml)	0.3579	0.0003
CEA (ng/ml)	YKL40 (ng/ml)	0.0890	0.3787
Periostin (ng/ml)	IgE (IU/ml)	0.3262	0.0009
Periostin (ng/ml)	YKL40 (ng/ml)	0.0108	0.9152
Periostin (ng/ml)	CEA (ng/ml)	0.3530	0.0003
Asthmatics; not on ICS (N=49)			
YKL40 (ng/ml)	IgE (IU/ml)	-0.1198	0.4123
CEA (ng/ml)	IgE (IU/ml)	0.3727	0.0084
CEA (ng/ml)	YKL40 (ng/ml)	0.1111	0.4471
Periostin (ng/ml)	IgE (IU/ml)	0.4236	0.0024
Periostin (ng/ml)	YKL40 (ng/ml)	0.0186	0.8989
Periostin (ng/ml)	CEA (ng/ml)	0.4033	0.0041
Asthmatics; on ICS (N=51)			
YKL40 (ng/ml)	IgE (IU/ml)	-0.2553	0.0706
CEA (ng/ml)	IgE (IU/ml)	0.2251	0.1123
CEA (ng/ml)	YKL40 (ng/ml)	0.1035	0.4699
Periostin (ng/ml)	IgE (IU/ml)	0.1974	0.1650
Periostin (ng/ml)	YKL40 (ng/ml)	0.0783	0.5849
Periostin (ng/ml)	CEA (ng/ml)	0.2197	0.1213
Asthmatics; IgE < 100 IU/ml (N=32)			
CEA (ng/ml)	YKL40 (ng/ml)	0.4003	0.0232
Periostin (ng/ml)	YKL40 (ng/ml)	0.3513	0.0487

Variable	by Variable	Spearman ρ	P-value
All subjects (Controls, N=48; Asthmatics, N=100)			
Periostin (ng/ml)	CEA (ng/ml)	0.1968	0.2802
Asthmatics; IgE > 100 IU/ml (N=68)			
CEA (ng/ml)	YKL40 (ng/ml)	0.0370	0.7647
Periostin (ng/ml)	YKL40 (ng/ml)	-0.1264	0.3043
Periostin (ng/ml)	CEA (ng/ml)	0.4145	0.0004

Spearman's rank order correlation, ρ , is indicated with associated p-values for the correlations. Highly significant p-values (<0.05) are indicated in **bold italics**.

[00159] Within the IL-13 signature, we observed several functional groups of multiple genes, including genes encoding protease inhibitors and genes expressed in mast cells and eosinophils, which may represent infiltration into and/or anatomic localization of those cells to bronchial epithelium. Greater than 90% of cells in each bronchial brushing sample were bronchial epithelial cells or goblet cells (mean 97%, median 98%, minimum 91%), but very small numbers of infiltrating "contaminant" cells with cell-specific gene expression patterns were detectable in the microarrays. Mast cell specific genes included tryptases (TPSAB1 [TPSD1] and TPSG1), CPA3, FCER1B, RGS13, and SLC18A2 [73, 74]. Also clustering tightly with mast cell genes was CNRIP1 (C2ORF32), a cannabinoid receptor-interacting GTPase. Given the well-established role of cannabinomimetics in the regulation of mast cell function [75], it is likely that CNRIP1 represents a mast cell-specific gene as well. Given the significant role of tissue-resident mast cells in allergic disease and the recent observation that the presence of IL-13 expressing mast cells in asthmatic endobronchial biopsy specimens is positively correlated with detectable levels of IL-13 in sputum [6], the high correlation between mast cell-specific genes and the IL-13 signature suggests that: 1) mast cells may be a significant source of IL-13 in the airway epithelium and 2) mast cell infiltration into airway epithelium may be a unique feature of the "IL-13 high" subset of asthmatics. Eosinophil specific genes include P2RY14 (GPR105) and ALOX15, although in Example 8 we described ALOX15 expression in alveolar macrophages from asthmatics.

[00160] Multiple probes corresponding to serine and cysteine protease inhibitors were present in the IL-13 signature, including Serpins B2 and B10, and cystatins (CST) 1, 2, and 4. SerpinB2 is a member of a large family of serine protease inhibitors encoded in a gene cluster on chromosome 18q21. Expression levels of Serpins B2 [8], B3, and B4 are induced in airway epithelial cells upon stimulation by recombinant IL-4 and IL-13 [7, 15]. Cystatins (CST) 1, 2, and 4 are members of a large family of cysteine protease inhibitors encoded in a gene cluster on chromosome 20p11. Several cystatins are expressed in bronchial epithelium [16]; CST4 has been identified at elevated levels in bronchoalveolar lavage fluid (BAL) of

asthmatics [17]; serum CST3 is elevated in asthmatics relative to healthy controls and its levels are decreased by ICS treatment [18]. As serpin and CST gene families are each colocalized on the chromosome, we explored whether any additional members of the serpin and cystatin gene families are co-regulated with those already identified. We performed hierarchical clustering of the microarray data across all subjects, restricted to serpin and cystatin gene families. We found that, out of over 40 protease inhibitor genes represented on the array, only serpins B2, B4, and B10; and cystatins 1, 2, and 4 were significantly co-regulated, with the highest expression levels occurring in asthmatics having the “IL-13 high” signature (Figure 2B and Table 12). As many aeroallergens possess protease activity and protease-activated receptors (PARs) are associated with the activation of allergic inflammatory cascades [76], the upregulation of protease inhibitors by Th2 cytokines may represent a compensatory response to protease-containing aeroallergens.

Table 12: Probe IDs of Serpin and CST genes used for clustering in Figure 2B. Probes are listed in order (top to bottom, left to right) found on heatmap at left of Figure 2B. Probes clustering with IL-13 signature genes are indicated in **bold**.

Probe ID	Gene Name	Probe ID	Gene Name	Probe ID	Gene Name
205075 at	SERPINF2	236599 at	SERPINE2	200986 at	SERPING1
206595 at	CST6	233968 at	CST11	1555551 at	SERPINB5
206325 at	SERPINA6	1554616 at	SERPINB8	233797 s at	CST11
206421 s at	SERPINB7	213874 at	SERPINA4	210140 at	CST7
227369 at	SERBP1	220627 at	CST8	209720 s at	SERPINB3
206034 at	SERPINB8	1568765 at	SERPINE1	209719 x at	SERPINB3
202376 at	SERPINA3	206386 at	SERPINA7	210413 x at	SERPINB4
207636 at	SERPINI2	202627 s at	SERPINE1	208531 at	SERPINA2
1552544 at	SERPINA12	1554491 a at	SERPINC1	209723 at	SERPINB9
231248 at	CST6	210076 x at	SERBP1	212190 at	SERPINE2
1553057 at	SERPINB12	217725 x at	SERBP1	211361 s at	SERPINB13
240177 at	CST3	217724 at	SERBP1	217272 s at	SERPINB13
202628 s at	SERPINE1	236449 at	CSTB	204855 at	SERPINB5
216258 s at	SERPINB13	207714 s at	SERPINH1	209725 at	UTP20
210049 at	SERPINC1	202283 at	SERPINF1	214539 at	SERPINB10
220626 at	SERPINA10	211474 s at	SERPINB6	204614 at	SERPINB2
209443 at	SERPINA5	209669 s at	SERBP1	208555 x at	CST2
209722 s at	SERPINB9	1556950 s at	SERPINB6	206224 at	CST1
202834 at	SERPINA8	228129 at	SERBP1	206994 at	CST4
205352 at	SERPINI1	201201 at	CSTB	211906 s at	SERPINB4
211362 s at	SERPINB13	213572 s at	SERPINB1	230318 at	SERPINA1
205576 at	SERPIND1	212268 at	SERPINB1	201360 at	CST3
1554386 at	CST9	1552463 at	SERPINB11	210466 s at	SERBP1
242814 at	SERPINB9	202833 s at	SERPINA1	204971 at	CSTA
239213 at	SERPINB1	211429 s at	SERPINA1		
230829 at	CST9L				

[00161] The mouse orthologue of CLCA1, mCLCA3 (also known as gob-5) has been previously identified as a gene associated with goblet cell metaplasia of airway epithelium and mucus production; both are induced by Th2 cytokines including IL-9 and IL-13 [12-14]. PRR4 is a member of a large gene family encoded in a cluster on chromosome 12p13. These genes encode proline-rich proteins, which are found in mucosal secretions including saliva and tears. Related, but non-orthologous proteins SPRR1a, 2a, and 2b have been identified in bronchial epithelium in a mouse model of asthma and are induced by IL-13 [19, 20]. Proline-rich proteins from the PRR/PRB family have been identified in bronchial secretions [21] and their expression has been documented in bronchial epithelium [16]. CCL26 (Eotaxin-3) is a well-documented IL-4 and IL-13 inducible eosinophil attracting chemokine in asthmatic airway epithelium [71]. CDH26 is a cadherin-like molecule of unknown function that has recently been identified in a microarray analysis of eosinophilic esophagitis [11]. That study identified several additional genes overlapping with our bronchial epithelial IL-13 signature including periostin, SerpinB4, and CCL26 [11]. As CDH26 is coregulated with cotaxins and overexpressed in diseases characterized by eosinophilic inflammation, it is tempting to speculate that CDH26 plays a role in eosinophil infiltration into mucosal tissues. Inducible nitric oxide synthase (iNOS) is associated with airway inflammation and is induced by IL-13 in human primary bronchial epithelial cell cultures [23]. The measurement of exhaled nitric oxide (eNO) is commonly used in the diagnosis and monitoring of asthma. Considered together, many of the genes described here as components of the IL-13 signature are highly consistent with in vitro and animal models of Th2 inflammation and play plausible roles in Th2-driven pathology in human asthma.

Table 13. Levels of serum biomarkers.

	Healthy Control (N=48)	Asthma (N=100)	P-value
IgE (IU/ml)	63 (0-590)	234 (1-2098)	<i><0.0001</i>
Periostin (ng/ml)	38 (0-139)	52 (0-117)	<i>0.03</i>
CEA (ng/ml)	<0.2 (<0.2-5.5)	2 (<0.2-21*)	<i><0.0001</i>
YKL-40 (ng/ml)	48 (18-265)	64 (19-494)	<i>0.0004</i>
Effect of inhaled corticosteroid treatment on serum biomarkers in asthmatics			

	No ICS (N=49)	ICS (N=51)	P-value
IgE (IU/ml)	322 (8-1395)	132 (1-2098)	<i>0.011</i>
Periostin (ng/ml)	54 (0-110)	48 (0-117)	0.07
CEA (ng/ml)	2.5 (<0.2-7.5)	1.9 (<0.2-21*)	<i>0.041</i>
YKL-40 (ng/ml)	62 (19-353)	72 (24-494)	0.30
Levels of serum biomarkers in asthmatics by IgE level category			
	IgE < 100 IU/ml (N=32)	IgE ≥ 100 IU/ml (N=68)	P-value
CEA (ng/ml)	1.6 (<0.2-7.5)	2.5 (<0.2-21*)	<i>0.031</i>
Periostin (ng/ml)	49 (0-117)	57 (0-112)	0.20
YKL-40 (ng/ml)	83 (19-494)	61 (23-290)	<i>0.01</i>

Values shown as median (range)

p-values are Wilcoxon rank rank sum

* 99/100 asthmatics had CEA values ≤ 7.5 ng/ml

[00162] CEACAM5 encodes a cell-surface glycoprotein found in many epithelial tissues and elevated serum CEACAM5 (carcinoembryonic antigen; CEA) is a well-documented systemic biomarker of epithelial malignancies and metastatic disease. Elevated CEA levels have been reported in a subset of asthmatics, with particularly high serum levels observed in asthmatics with mucoid impaction [75]. Intriguingly, while the upper limit of normal for serum CEA is in the 2.5-3 ng/ml range, the lower limit for suspicion of malignancy is 10 ng/ml. In our analyses, we find that over 95% of healthy controls had CEA levels below 3 ng/ml while 1/3 of asthmatics had CEA levels between 3 and 7.5 ng/ml, and of these, the vast majority had serum IgE levels above 100 IU/ml. This suggests that a robust window of detection for CEA may be present in asthmatics with Th2-driven airway inflammation. Periostin has been described as an IL-4 and IL-13 inducible gene in asthmatic airways [7-9, 77] as a gene upregulated in epithelial-derived cancers that may be associated with invasiveness and extracellular matrix change [64-67], and whose serum protein levels are detectable and elevated in some cancers [68-70]. As it may play a role in eosinophilic tissue

infiltration in eosinophilic esophagitis [11, 77], periostin could be an important factor in, and biomarker of, eosinophilic diseases such as Th2-driven asthma.

[00163] The standard of care for bronchial asthma that is not well-controlled on symptomatic therapy (i.e. β -agonists) is inhaled corticosteroids (ICS). In mild-to-moderate asthmatics with elevated levels of IL-13 in the airway [6] and eosinophilic esophagitis patients with elevated expression levels of IL-13 in esophageal tissue [11], ICS treatment substantially reduces the level of IL-13 and IL-13-induced genes in the affected tissues. In airway epithelium of asthmatics after one week of ICS treatment and in cultured bronchial epithelial cells, we have shown that corticosteroid treatment substantially reduces IL-13-induced expression levels of periostin, serpinB2, and CLCA1 [8]. Further examination of the genes listed in Table 9 revealed that, in the 19 subjects in our study who received one week of ICS treatment prior to a second bronchoscopy, the vast majority of IL-13 signature genes was significantly downregulated by ICS treatment in asthmatic bronchial airway epithelium. This downregulation could be the result of ICS-mediated reduction of IL-13 levels, ICS-mediated reduction of target gene expression, or a combination of the two. In severe asthmatics who are refractory to ICS treatment, a similar fraction of subjects (approximately 40%) was found to have detectable sputum IL-13 levels to that seen in mild, ICS-naïve asthmatics [6], which is comparable to the fraction of subjects with the IL-13 signature observed in this study. This observation suggests that, although the IL-13 signature is significantly downregulated by ICS treatment in the mild-moderate, ICS-responsive asthmatics examined in the present study, it may still be present in severe steroid-resistant asthmatics. Similar observations have been reported for eosinophilic inflammation in bronchial biopsies [78] and persistence of IL-4 and IL-5 expressing cells in BAL [79] of steroid-refractory asthmatics. There is currently a large number of biological therapeutics in clinical development directed against IL-13 or related factors in Th2 inflammation [50, 80], including, without limitation, those described herein. Our findings suggest that only a fraction of steroid-naïve mild-to-moderate asthmatics may have activity of this pathway, and given its susceptibility to ICS treatment, it is likely that a smaller fraction of moderate-to-severe, steroid-refractory asthmatics has activity of this pathway. Therefore, biomarkers that identify asthmatics likely to have IL-13 driven inflammation in their airways may aid in the identification and selection of subjects most likely to respond to these experimental targeted therapeutics.

CLAIMS

We claim:

1. A method of diagnosing an asthma subtype in a patient comprising measuring the gene expression of any one or combination of genes selected from the group of consisting of POSTN, CST1, CST2, CCL26, CLCA1, PRR4, PRB4, SERPINB2, CEACAM5, iNOS, SERPINB4, CST4, and SERPINB10, wherein elevated expression levels of any one, combination or all of said genes is indicative of the asthma subtype.
2. The method according to claim 1, further comprising the genes PRB4, TPSD1, TPSG1, MFSD2, CPA3, GPR105, CDH26, GSN, C2ORF32, TRACH2000196 (TMEM71), DNAJC12, RGS13, SLC18A2, SH3RF2, FCER1B, RUNX2, PTGS1, and ALOX15.
3. The method according to claim 1, wherein gene expression is measured by assaying for protein or mRNA levels.
4. The method according to claim 3, wherein the mRNA levels are measured by using a PCR method or a microarray chip.
5. The method according to claim 4, wherein the PCR method is qPCR.
6. The method according to claim 3, wherein the mRNA levels of the gene of interest relative to a control gene mRNA levels greater than 2.5 fold is indicative of the asthma subtype.
7. A method of diagnosing an asthma subtype in a patient comprising measuring any one of the biomarkers from a patient sample selected from the group consisting of: serum total IgE levels, serum CEA levels, serum periostin levels, peripheral blood eosinophils and bronchoalveolar lavage (BAL) eosinophils, wherein elevated levels of CEA, serum periostin, peripheral blood eosinophils and bronchoalveolar lavage (BAL) eosinophils is indicative of the asthma subtype.
8. The method according to claim 7, wherein an IgE level greater than 100IU/ml is indicative of the asthma subtype.

9. The method according to claim 7, wherein a peripheral blood eosinophil level greater than $0.14 \times 10^9/L$ is indicative of the asthma subtype.
10. A method of diagnosing an asthma subtype in a patient comprising measuring the ratio of Muc5AC:MUC5B mRNA or the ratio of Muc5AC:MUC5B protein from a sample of an asthma patient, wherein a ratio greater than 25 is indicative of the asthma subtype.
11. The method according to claim 10, wherein the sample is obtained from an epithelial brushing.
12. The method according to claim 10, wherein the sample comprises airway epithelial cells.
13. A method of treating asthma comprising administering a therapeutic agent to a patient expressing elevated levels of any one or combination of the genes selected from the group consisting of POSTN, CST1, CST2, CCL26, CLCA1, PRR4, PRB4, SERPINB2, CEACAM5, iNOS, SERPINB4, CST4, and SERPINB10.
14. The method according to claim 13, further comprising the genes PRB4, TPSD1, TPSG1, MFSD2, CPA3, GPR105, CDH26, GSN, C2ORF32, TRACH2000196 (TMEM71), DNAJC12, RGS13, SLC18A2, SH3RF2, FCER1B, RUNX2, PTGS1, and ALOX15.
15. A method of treating asthma comprising administering a therapeutic agent to a patient expressing elevated levels of serum total IgE, serum CEA, serum periostin, peripheral blood eosinophils and/or bronchoalveolar lavage (BAL) eosinophils.
16. A method of treating asthma comprising administering a therapeutic agent to a patient having a ratio of Muc5AC:MUC5B mRNA or ratio of Muc5AC:MUC5B protein greater than 25 in a patient sample.
17. The method according to any one of claims 13-16, wherein the patient to be treated is a mild-to-moderate, steroid-naïve asthma patient.

18. The method according to any one of claims 13-16, wherein the patient to be treated is a moderate-to-severe, steroid-resistant asthma patient.
19. The method according to any one of claims 13-16, wherein the patient has asthma induced by the TH2 pathway.
20. The method according to any one of claims 13-16, wherein the patient has been diagnosed according to the method of any one of the aforementioned claims.
21. The method according to any one of claims 13-16, wherein the therapeutic agent is selected from the group consisting of an agent that binds to a target selected from the group consisting of: IL-9, IL-5, IL-13, IL-4, OX40L, TSLP, IL-25, IL-33 and IgE; and receptors such as: IL-9 receptor, IL-5 receptor, IL-4receptor alpha, IL-13receptoralpha1 and IL-13receptoralpha2, OX40, TSLP-R, IL-7Ralpha, IL17RB, ST2, CCR3, CCR4, CRTH2, FcepsilonRI and FcepsilonRII/CD23.
22. The method according to any one of claims 13-16, wherein the therapeutic agent is an immunoadhesin, a peptibody or an antibody.
23. A method of treating asthma comprising administering a therapeutic agent to an asthma patient not expressing elevated levels of any one or combination of the genes selected from the group consisting of POSTN, CST1, CST2, CCL26, CLCA1, PRR4, PRB4, SERPINB2, CEACAM5, iNOS, SERPINB4, CST4, and SERPINB10.
24. The method according to claim 23, further comprising the genes PRB4, TPSD1, TPSG1, MFSD2, CPA3, GPR105, CDH26, GSN, C2ORF32, TRACH2000196 (TMEM71), DNAJC12, RGS13, SLC18A2, SH3RF2, FCER1B, RUNX2, PTGS1, and ALOX15.
25. A method of treating asthma comprising administering a therapeutic agent to an asthma patient not expressing elevated levels of serum total IgE levels, serum CEA levels, serum periostin levels, peripheral blood eosinophils and/or bronchoalveolar lavage (BAL) eosinophils.

26. A method of treating asthma comprising administering a therapeutic agent to an asthma patient not having a Muc5AC:MUC5B mRNA or protein ratio greater than 25 in a patient sample.
27. The method according to claim 26, wherein the therapeutic agent is an IL-17 pathway inhibitor.
28. A kit for diagnosing an asthma subtype in a patient comprising (1) one or more nucleic acid molecules that hybridize with a gene, wherein the gene is selected from the group of consisting of POSTN, CST1, CST2, CCL26, CLCA1, PRR4, PRB4, SERPINB2, CEACAM5, iNOS, SERPINB4, CST4, and SERPINB10 and (2) instructions for measuring the expression levels of the gene from a patient sample, wherein the elevated expression levels of any one, combination or all of said genes is indicative of the asthma subtype.
29. The kit according to claim 28, further comprising a gene selected from the group consisting of: PRB4, TPSD1, TPSG1, MFSD2, CPA3, GPR105, CDH26, GSN, C2ORF32, TRACH2000196 (TMEM71), DNAJC12, RGS13, SLC18A2, SH3RF2, FCER1B, RUNX2, PTGS1, and ALOX15.
30. The kit according to claim 28, wherein gene expression is measured by assaying for mRNA levels.
31. The kit according to claim 30, wherein the assay comprises a PCR method or the use of a microarray chip.
32. The kit according to claim 31, wherein the PCR method is qPCR.
33. The kit according to claim 30, wherein the mRNA levels of the gene of interest relative to a control gene mRNA level greater than 2.5 fold is indicative of the asthma subtype.
34. A kit for diagnosing an asthma subtype in a patient comprising (1) one or more protein molecules that bind to a protein selected from the group of consisting of POSTN, CST1, CST2, CCL26, CLCA1, PRR4, PRB4, SERPINB2, CEACAM5, iNOS, SERPINB4, CST4, and SERPINB10 and (2) instructions for measuring the expression levels of the protein from

a patient sample, wherein the elevated expression levels of any one, combination or all of said proteins is indicative of the asthma subtype.

35. The kit according to claim 10, further comprising a protein is selected from the group consisting of: PRB4, TPSD1, TPSG1, MFSD2, CPA3, GPR105, CDH26, GSN, C2ORF32, TRACH2000196 (TMEM71), DNAJC12, RGS13, SLC18A2, SH3RF2, FCER1B, RUNX2, PTGS1, and ALOX15.

36. The kit according to claim 34, wherein the assay comprises the use of a microarray chip comprising the protein molecules.

37. A kit for diagnosing an asthma subtype in a patient comprising instructions for measuring any one of the biomarkers from a patient sample selected from the group consisting of: serum total IgE levels, serum CEA levels, serum periostin levels, peripheral blood eosinophils and bronchoalveolar lavage (BAL) eosinophils, wherein elevated levels of CEA, serum periostin, peripheral blood eosinophils and bronchoalveolar lavage (BAL) eosinophils.

38. The kit according to claim 37, wherein an IgE level greater than 100IU/ml is indicative of the asthma subtype.

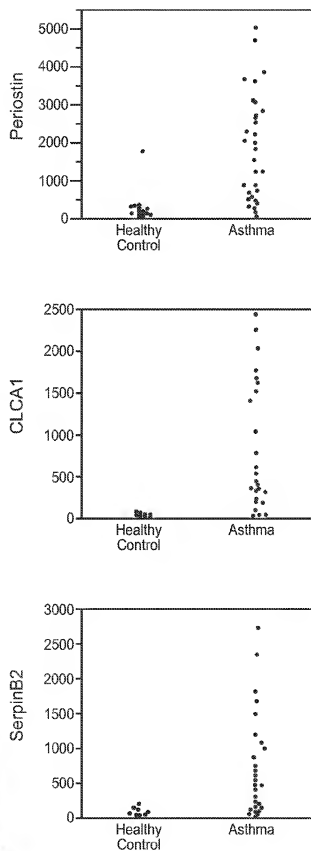
39. The kit according to claim 37, wherein a peripheral blood eosinophil level greater than $0.14 \times 10^9/L$ is indicative of the asthma subtype.

40. A kit for diagnosing an asthma subtype in a patient comprising instructions for measuring the ratio of Muc5AC:MUC5B mRNA or protein from a sample of an asthma patient, wherein a ratio greater than 25 is indicative of the asthma subtype.

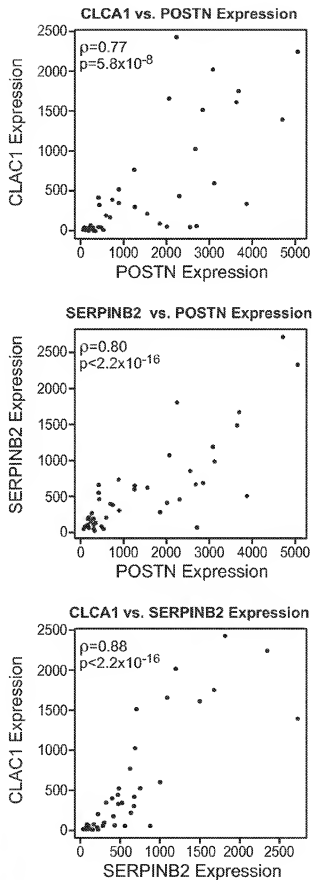
41. The kit according to claim 40, wherein the sample is obtained from an epithelial brushing.

42. The kit according to claim 40, wherein the sample comprises airway epithelial cells.

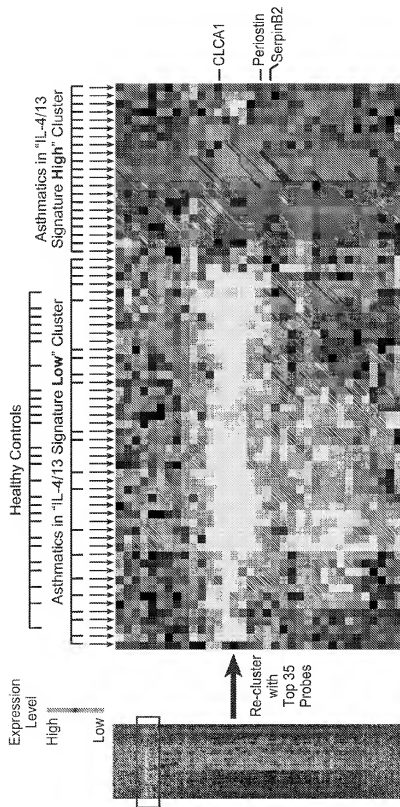
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FIG. 1A

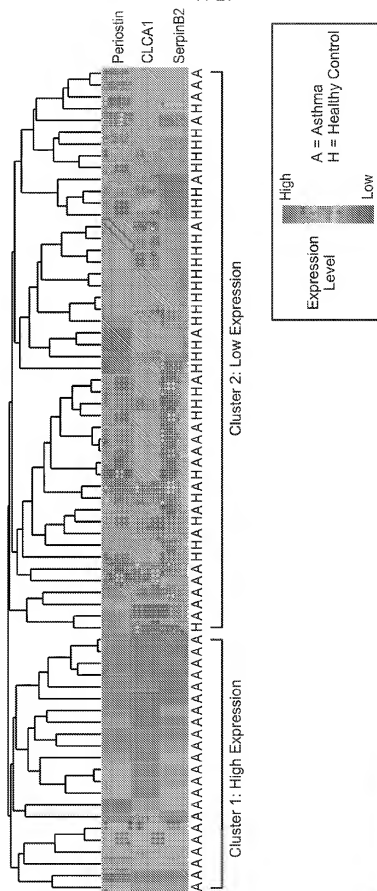
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FIG. 1B

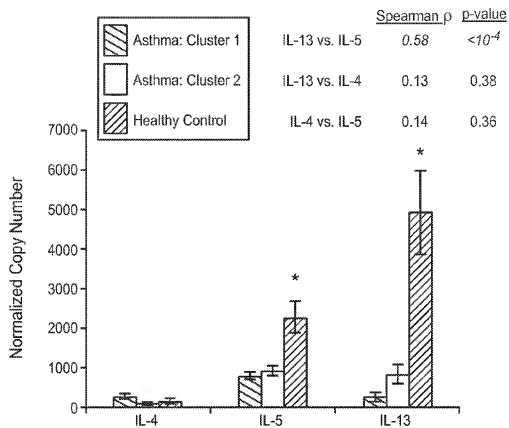
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**FIG. 1C**

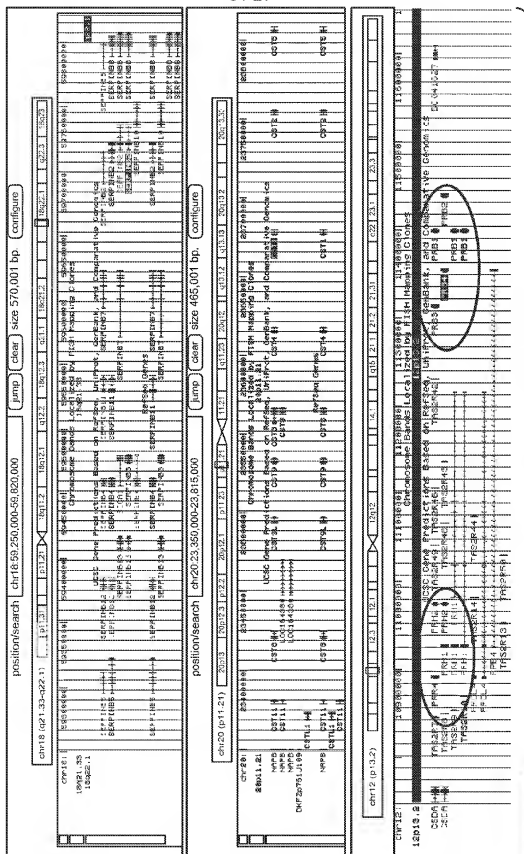
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**FIG. 1D**

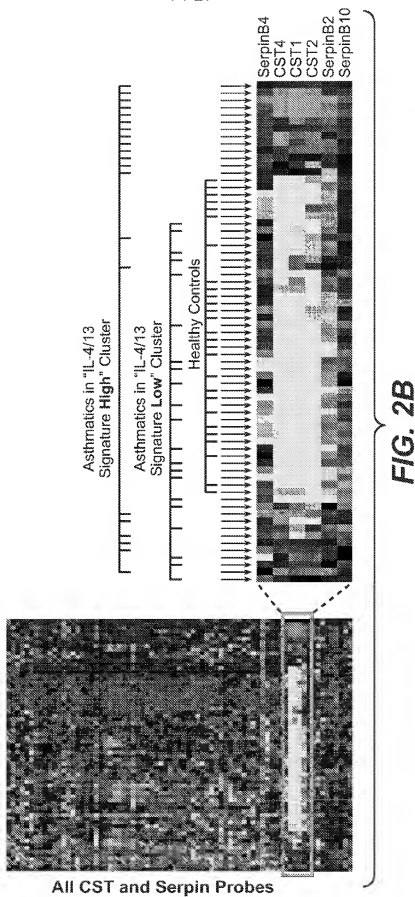
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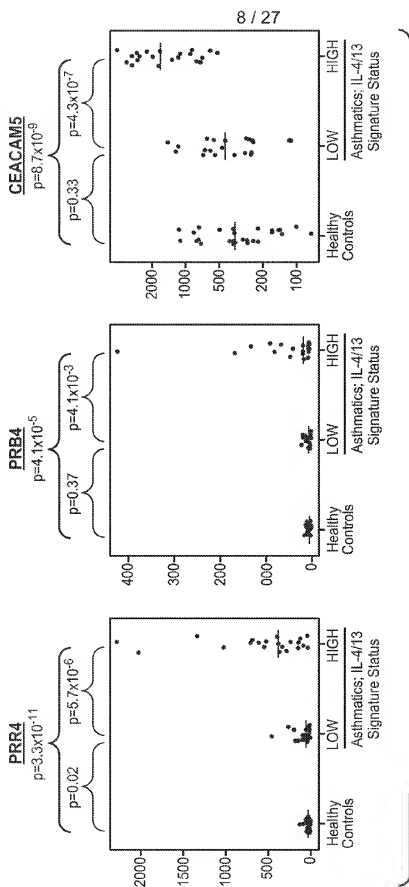
**FIG. 1E**

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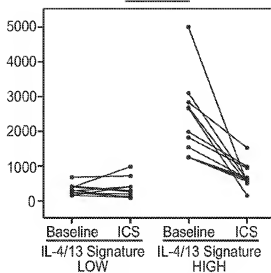
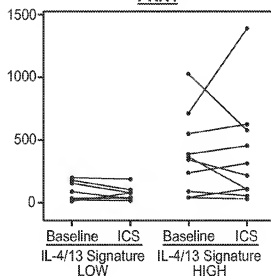
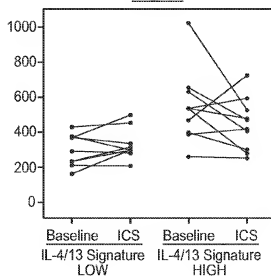


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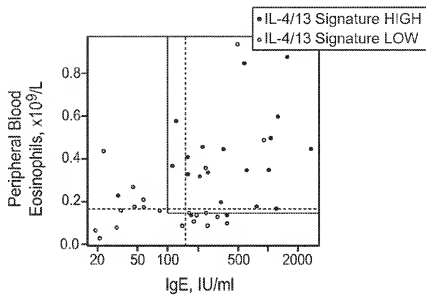




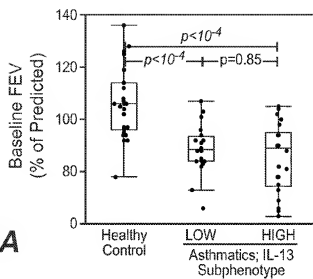
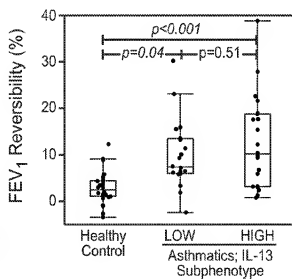
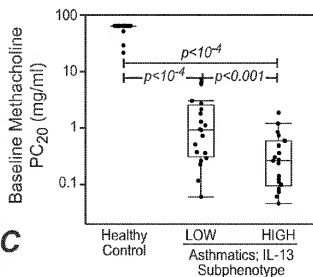
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Periostin**FIG. 3A**PRR4**FIG. 3B**RUNX2**FIG. 3C**

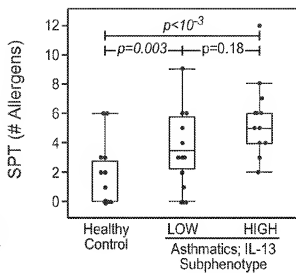
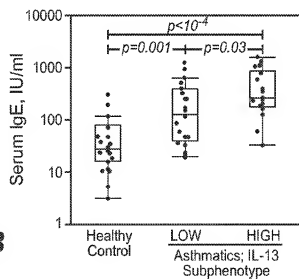
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**FIG. 4**

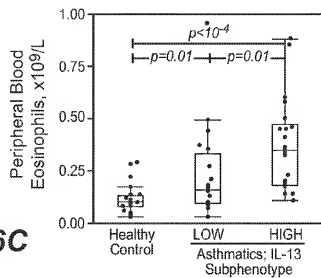
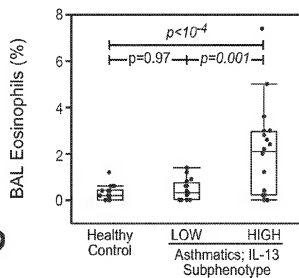
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**FIG. 5A****FIG. 5B****FIG. 5C**

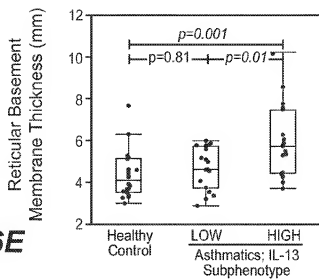
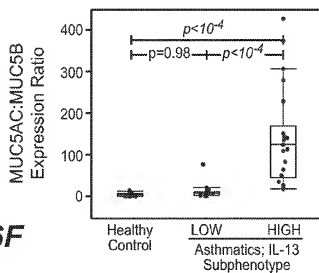
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**FIG. 6A****FIG. 6B**

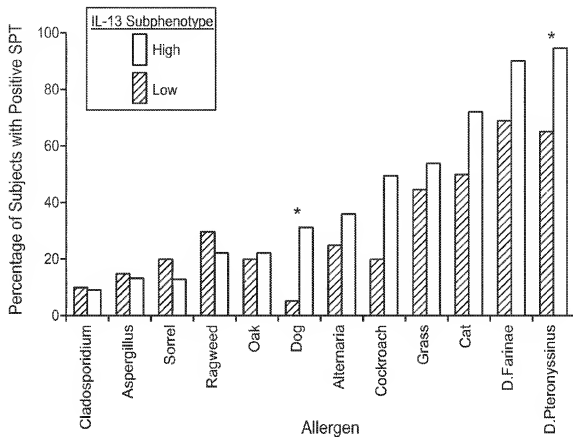
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**FIG. 6C****FIG. 6D**

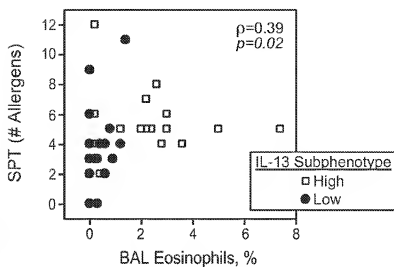
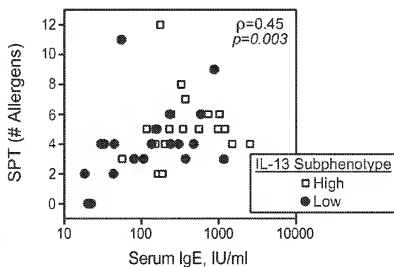
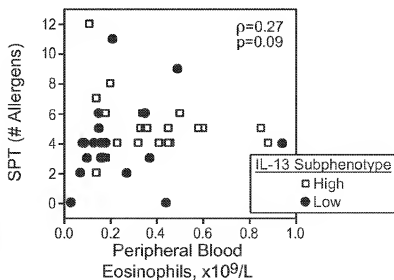
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**FIG. 6E****FIG. 6F**

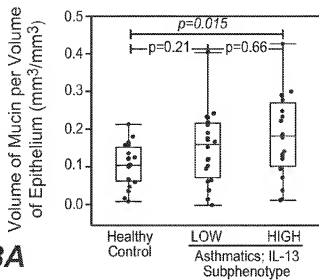
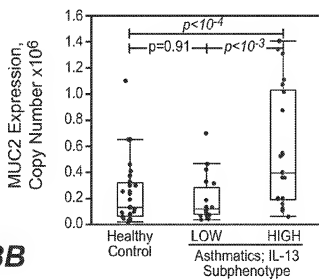
15 / 27

**FIG. 7A**

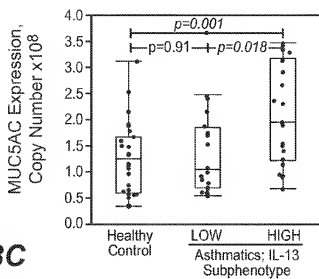
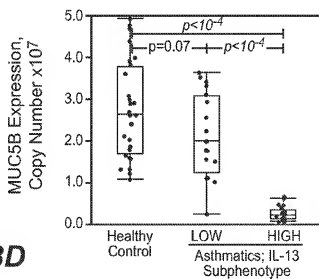
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**FIG. 7B****FIG. 7C****FIG. 7D**

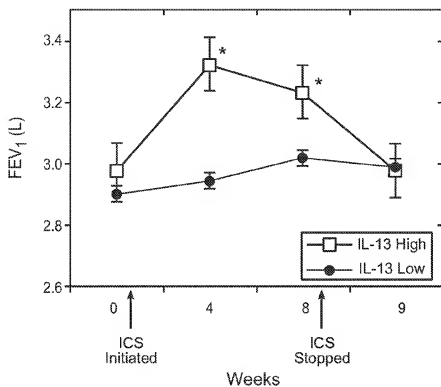
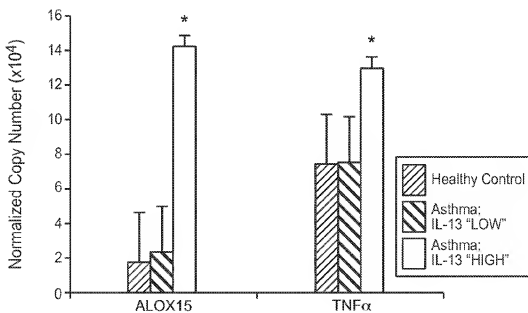
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**FIG. 8A****FIG. 8B**

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**FIG. 8C****FIG. 8D**

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**FIG. 9A****FIG. 10**

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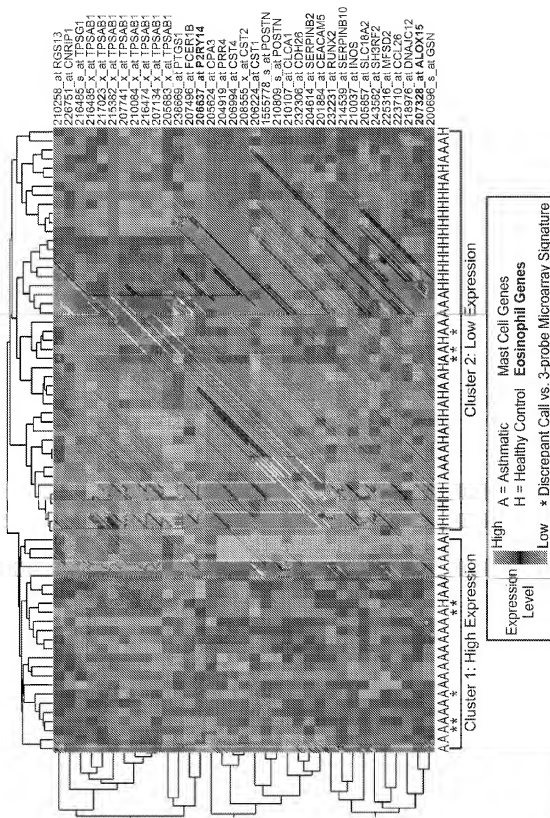
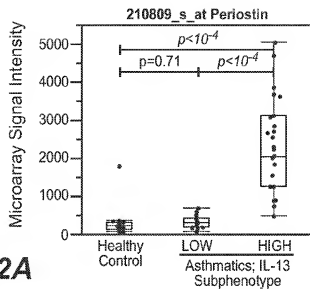
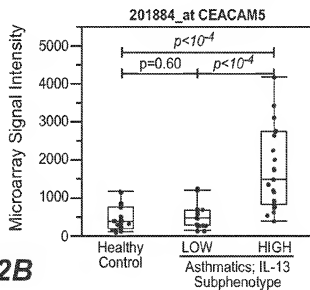
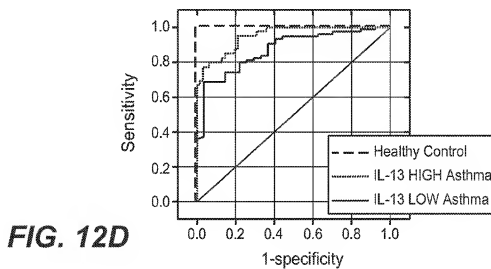
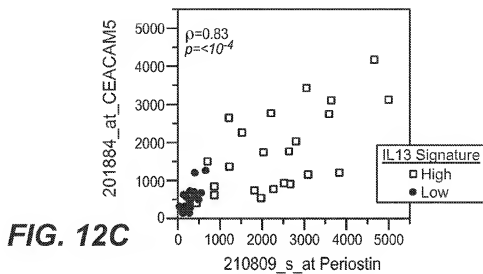


FIG. 11

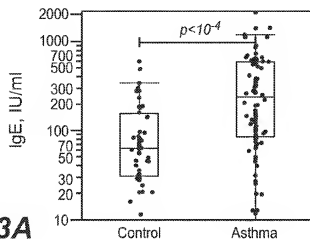
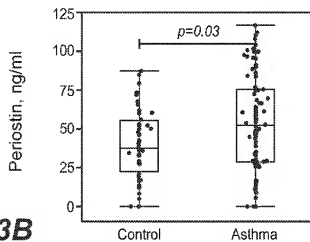
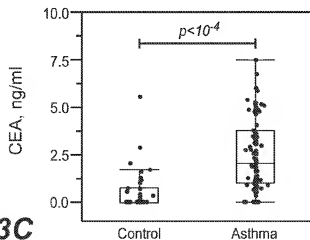
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**FIG. 12A****FIG. 12B**

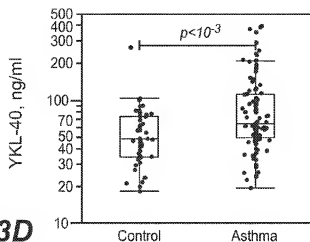
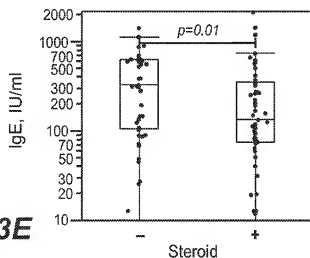
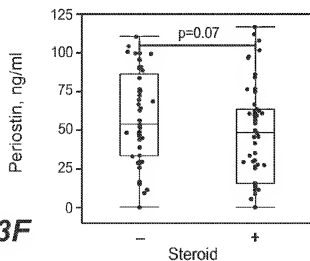
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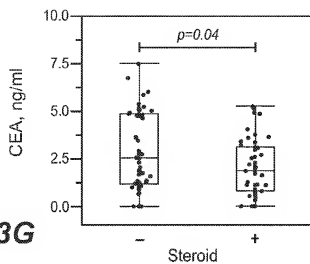
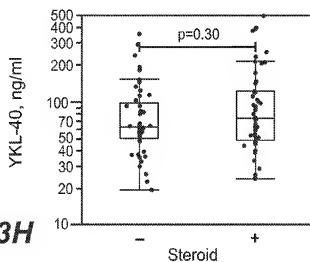
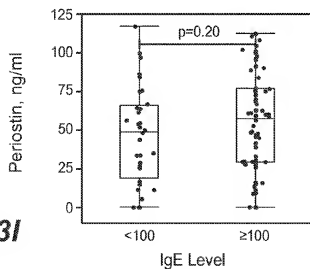
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**FIG. 13A****FIG. 13B****FIG. 13C**

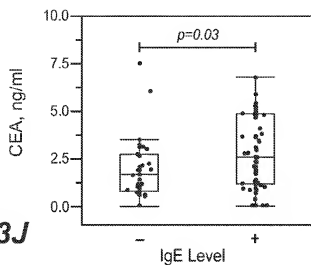
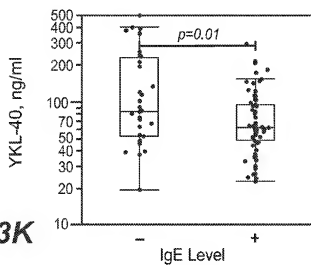
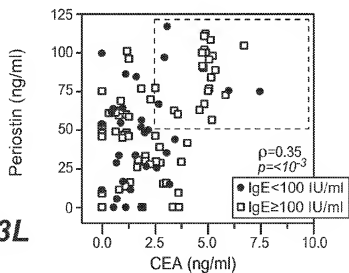
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**FIG. 13D****FIG. 13E****FIG. 13F**

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**FIG. 13G****FIG. 13H****FIG. 13I**

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**FIG. 13J****FIG. 13K****FIG. 13L**

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2009/039033

A. CLASSIFICATION OF SUBJECT MATTER

INV. C12Q1/68

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
C12Q

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, BIOSIS, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	SIMPSON JODIE L ET AL: "Inflammatory subtypes in asthma: assessment and identification using induced sputum." RESPIROLOGY (CARLTON, VIC.) JAN 2006, vol. 11, no. 1, January 2006 (2006-01), pages 54-61, XP002532411 ISSN: 1323-7799 abstract	7
X	WENZEL SALLY E: "Asthma: defining of the persistent adult phenotypes." LANCET 26 AUG 2006, vol. 368, no. 9537, 26 August 2006 (2006-08-26), pages 804-813, XP002532409 ISSN: 1474-547X the whole document	7-25
-/-		

☒ Further documents are listed in the continuation of Box C.

☒ See patent family annex.

* Special categories of cited documents:

- *A* document defining the general state of the art which is not considered to be of particular relevance
- *E* earlier document but published on or after the international filing date
- *L* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- *O* document referring to an oral disclosure, use, exhibition or other means
- *P* document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

X document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

Y document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

A document member of the same patent family

Date of the actual completion of the international search

18 August 2009

Date of mailing of the international search report

27/08/2009

Name and mailing address of the ISA/
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Authorized officer

Cornelis, Karen

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US2009/039033

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WOODRUFF PRESCOTT G ET AL: "Genome-wide profiling identifies epithelial cell genes associated with asthma and with treatment response to corticosteroids" PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF USA, NATIONAL ACADEMY OF SCIENCE, WASHINGTON, DC, US, vol. 104, no. 40, 1 October 2007 (2007-10-01), pages 15858-15863, XP002532408 ISSN: 0027-8424 the whole document	1-7,13, 28-36
A	IZUHARA KENJI ET AL: "Microarray-based identification of novel biomarkers in asthma." ALLERGOLOGY INTERNATIONAL : OFFICIAL JOURNAL OF THE JAPANESE SOCIETY OF ALLERGOLOGY DEC 2006, vol. 55, no. 4, December 2006 (2006-12), pages 361-367, XP002532410 ISSN: 1323-8930 the whole document	1-42
Y	TAKAYAMA ET AL: "Periostin: A novel component of subepithelial fibrosis of bronchial asthma downstream of IL-4 and IL-13 signals" JOURNAL OF ALLERGY AND CLINICAL IMMUNOLOGY, MOSBY - YEARLY BOOK, INC, US, vol. 118, no. 1, 1 July 2006 (2006-07-01), pages 98-104, XP005610109 ISSN: 0091-6749 the whole document	7-9, 15-22
A	WO 03/021261 A (DECODE GENETICS EHF [IS]; HAKONARSON HAKON [IS]) 13 March 2003 (2003-03-13) the whole document	1-42
X,P	STANKOVIC KONSTANTINA M ET AL: "Gene expression profiling of nasal polyps associated with chronic sinusitis and aspirin-sensitive asthma" LARYNGOSCOPE, TRIOLOGICAL FOUNDATION, ST LOUIS, US, vol. 118, no. 5, 1 May 2008 (2008-05-01), pages 881-889, XP009113976 ISSN: 0023-852X the whole document	1-6

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INTERNATIONAL SEARCH REPORT

International application No

PCT/US2009/039033

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	<p>TILMAN GAÅ LLE ET AL: "Human periostin gene expression in normal tissues, tumors and melanoma: evidences for periostin production by both stromal and melanoma cells"</p> <p>MOLECULAR CANCER, BIOMED CENTRAL, LONDON, GB,</p> <p>vol. 6, no. 1,</p> <p>17 December 2007 (2007-12-17), page 80,</p> <p>XP021036924</p> <p>ISSN: 1476-4598</p> <p>abstract</p> <p>-----</p>	1
X	<p>LIAO QUAN ET AL: "Combined detection of serum tumor markers for differential diagnosis of solid lesions located at the pancreatic head."</p> <p>HEPATOBIILIARY & PANCREATIC DISEASES INTERNATIONAL : HBPDI INT DEC 2007,</p> <p>vol. 6, no. 6, December 2007 (2007-12),</p> <p>pages 641-645, XP002541522</p> <p>ISSN: 1499-3872</p> <p>abstract</p> <p>-----</p>	37-39
X	<p>SASAKI HIDEFUMI ET AL: "Elevated serum periostin levels in patients with bone metastases from breast but not lung cancer."</p> <p>BREAST CANCER RESEARCH AND TREATMENT FEB 2003,</p> <p>vol. 77, no. 3, February 2003 (2003-02),</p> <p>pages 245-252, XP002541523</p> <p>ISSN: 0167-6806</p> <p>abstract</p> <p>-----</p>	37-39
Y		7-9, 15-22
X	<p>SANZ M L ET AL: "Serum eosinophil peroxidase (EPO) levels in asthmatic patients."</p> <p>ALLERGY APR 1997,</p> <p>vol. 52, no. 4, April 1997 (1997-04),</p> <p>pages 417-422, XP002541524</p> <p>ISSN: 0105-4538</p> <p>abstract</p> <p>-----</p>	7-9, 15-18, 37-39
	-/-	

INTERNATIONAL SEARCH REPORT

International application No

PCT/US2009/039033

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	<p>WENZEL S E ET AL: "Bronchoscopic evaluation of severe asthma. Persistent inflammation associated with high dose glucocorticoids." AMERICAN JOURNAL OF RESPIRATORY AND CRITICAL CARE MEDICINE SEP 1997, vol. 156, no. 3 Pt 1, September 1997 (1997-09), pages 737-743, XP002541525 ISSN: 1073-449X abstract</p>	<p>7-9, 15-18, 37-39</p>
X	<p>VIGNOLA A M ET AL: "Airway inflammation in mild intermittent and in persistent asthma." AMERICAN JOURNAL OF RESPIRATORY AND CRITICAL CARE MEDICINE FEB 1998, vol. 157, no. 2, February 1998 (1998-02), pages 403-409, XP002541526 ISSN: 1073-449X the whole document</p>	<p>7-9,37</p>

INTERNATIONAL SEARCH REPORTInternational application No.
PCT/US2009/039033**Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)**

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of additional fees.
3. ☒ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
1-9, 13-15, 17-24, 28-39(partially)
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
- ☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
- ☒ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/SA 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. claims: 1-6, 13-14, 17-24, 28-36(partially)

A method of diagnosing an asthma subtype by measuring gene expression of POSTN, or a combination of POSTN and one or more genes of claim 1. A method of treating asthma by administering a therapeutic agent to a patient expressing elevated levels of POSTN or a combination of POSTN and one of the genes of claim 13, a method of treating asthma comprising administering a therapeutic agent to a patient not expressing elevated levels of POSTN or a combination of POSTN and any other gene of claim 23.

A kit for diagnosing an asthma subtype comprising a nucleic acid molecule that hybridises with POSTN or a protein molecule that binds to POSTN.

2-13. claims: 1-6, 13-14, 17-24, 28-36(partially)

A method of diagnosing an asthma subtype by measuring gene expression of CST1, or a combination of CST1 and one or more genes of claim 1. A method of treating asthma by administering a therapeutic agent to a patient expressing elevated levels of CST1 or a combination of CST1 and one of the genes of claim 13, a method of treating asthma comprising administering a therapeutic agent to a patient not expressing elevated levels of CST1 or a combination of CST1 and any other gene of claim 23.

A kit for diagnosing an asthma subtype comprising a nucleic acid molecule that hybridises with CST1 or a protein molecule that binds to CST1.

Analogous for CST2, CCL26, CLA1, PRR4, PRB4, serpinB2, CEACAM5, iNOS, serpinB4, CST4 and SerpinB10.

14. claims: 7-9, 15, 17-22, 25, 37-39(partially)

A method for diagnosing an asthma subtype measuring IgE, a kit.

15-18. claims: 7-9, 15, 17-22, 25, 37-39(partially)

A method to diagnose asthma subtype by measuring serum CEA, method of treatment, kit. Analogously for serum periostin, peripheral blood eosinophils, BAL eosinophils

19. claims: 10-12, 16, 26-27, 40-42(completely); 17-22(partially)

method of diagnosing asthma subtype measuring ratio Muc5AC:Muc5B mRNA or protein, a kit.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/US2009/039033

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 03021261	A	13-03-2003	AT 406575 T 15-09-2008
			CA 2457476 A1 13-03-2003
			DK 1428023 T3 19-01-2009
			EP 1428023 A2 16-06-2004
			JP 2005502345 T 27-01-2005
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			US 2003113831 A1 19-06-2003
